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TAINTING AND TOXICITY IN SEA SCALLOPS (Placopecten magellanicus) EXPOSED TO THE WATER-SOLUBLE FRACTION OF SCOTIAN SHELF NATURAL GAS CONDENSATE

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ABSTRACT

A natural gas liquids (condensate) blowout in 1984 off Sable Island raised concerns about the possibility of tainting (development of an off-flavour in the edible tissue of commercial species) and toxic effects, depending on conditions, if there are any condensate blowouts in the future. The present study was concerned with tainting and toxicity in sea scallops (Placopecten magellanicus) exposed to the water-soluble fraction (WSF) of Scotian Shelf condensate. Scallops are presently harvested near potential natural gas fields off Sable Island.

The objectives of the study were to assess the chemical properties of Scotian Shelf condensate, to determine the acute toxicity of condensate in scallops, and to determine the potential for tainting in scallops exposed to the WSF of Scotian Shelf condensate.

Scallops with a shell height exceeding 8 cm were collected from the Bay of Fundy. They were kept at holding facilities outside Halifax. Experiments were conducted at Dalhousie University and the Bedford Institute of Oceanography. Water-soluble fraction was prepared in an 1,100 L stainless steel mixing vessel at a mixing ratio of 1:299 (condensate:-seawater) immediately prior to use in tainting and toxicity exposures. Toxicity tests were conducted in static 20 L containers using different dilutions of the WSF. Observations included delayed mortalities within 5 days of the exposures. Exposures for tainting evaluation involved a continuous flow of WSF and diluent seawater, allowing maintenance of relatively stable hydrocarbon levels. These were monitored frequently using a fluorometer. Analytical results were calibrated with gas chromatography. Tainting exposures ranged from 3 h to 96 h. Depuration was also examined in scallops exposed to WSF for 24 and 96 h. The Murray microextraction procedure was used for extraction of hydrocarbons from samples. Condensate, exposure water, and scallop adductor muscles were analyzed by gas chromatography and by gas chromatography/mass spectrometry. Tainting was evaluated by the taste panel method involving triangle tests with control samples.

The major components of Scotian Shelf condensate were aromatic hydrocarbons, with benzene, toluene and xylenes dominating. There were moderate levels of naphthalene and its methyl and dimethyl derivatives. The hydrocarbon concentration of the WSF ranged from 42 to 56 ppm. The WSF was dominated by low molecular weight aromatics. There were fewer n-alkanes in the WSF than in the parent condensate.

The 96-h LC50 for sea scallops exposed to the condensate WSF was 17.4 ppm (initial measured concentration). When delayed mortalities were included, the 96-h LC50 was 12.5 ppm.

Tainting was evident in scallops exposed for 3 h to 2.1 - 4.8 ppm WSF. Tainting was also evident in scallops exposed for 24 h to 3.8 ppm, but not when exposed to 0.8 ppm. Scallops exposed for 96 h to 0.8 ppm

were significantly tainted, but those exposed to 0.6 ppm were not. Scallops which were significantly tainted after 24 h exposure to a WSF concentration of 2.7 ppm were free of the taint after 24 h in clean seawater. On the other hand, scallops which were tainted after 96 h exposure to a WSF concentration of 0.9 ppm were only free of a taint after between 96 and 264 h in clean seawater.

Scallops exposed for 24 h to a WSF concentration of $\geq$ 2.7 ppm showed significant uptake of hydrocarbons in the adductor muscle. Those with a concentration of greater than 15.6 ppm in the adductor muscle were significantly tainted. Scallops exposed to <1 ppm WSF did not show significant uptake of hydrocarbons and were not tainted. Analysis indicated rapid loss of hydrocarbons from the adductor muscle within 24 h. The major hydrocarbons in tainted adductor muscles were toluene and xylenes. Naphthalene, methyl and dimethylnaphthalene also accumulated in the adductor muscle.

The analytical results indicated only minimal weathering of the condensate prior to its use in the tainting and toxicity exposures. The condensate had relatively high solubility compared to various crude oils. However, it was also very volatile, with 90% evaporating within 24 h in the toxicity exposures. There was minimal loss of the condensate during the tainting exposures because of continuous input of the WSF, which allowed maintenance of relatively stable hydrocarbon levels. In the analysis of the WSF, the hydrocarbon recoveries were relatively low, but there was good repeatibility, allowing confidence in the data for the exposure water. Recoveries for hydrocarbons in the adductor muscle were very low, so that the data for hydrocarbon levels in the adductor muscle can only be considered semi-quantitative.

The WSF of Scotian Shelf condensate was similar to the WSFs of various crude oils, indicating the relatively conservative nature of the truly dissolved fraction of oil. The toxicity tests indicated that scallops are less sensitive to the toxic components of condensate than various fish species which have been tested. However, scallops are at least twice as sensitive as sea urchins.

The tainting threshold for scallops in 3-96 h exposures to condensate WSF is 0.8 ppm. Increasing the exposure time for a given exposure level tended to increase the degree of tainting, especially near the threshold level. The tainting threshold for the WSF of Scotian Shelf condensate is similar to that for the WSF of Ninian crude oil (0.5 ppm). For scallops exposed to low levels of WSF (<1 ppm), the level of accumulation of hydrocarbons in the adductor muscle was only 1.6-1.8 x the exposure level. At higher exposure levels (2.7-3.8 ppm), the accumulation of hydrocarbons was 5.9-12.9 x the exposure level and these scallops were significantly tainted. Although toluene and xylenes were the most significant components in exposed adductor muscles, they only accumulated at a level reflecting their proportion in the WSF, indicating no selective uptake or retention of these light aromatic compounds. On the other hand, there was selective retention of alkylated benzenes, naphthalene, and alkylated naphthalenes.

Toluene and xylenes were implicated in tainting in sea scallops. Higher levels of alkylated benzenes, naphthalene, and alkylated naphthalenes may also contribute to tainting; however, they do not cause tainting when present in the adductor muscle at low levels. The rapid depuration of the tainted scallop adductor muscles was probably related to the rapid reduction in the concentration of the lighter aromatic compounds. After 24 h of depuration, only 8% of the accumulated hydrocarbons remained.

The mixing ratio data indicate that a true WSF comprises no more than 2-8% of the volume of the whole condensate, depending on the mixing ratio. With a tainting threshold of 0.8 ppm, the concentration of whole condensate in the water column would have to be sustained at at least 10 ppm to cause tainting in sea scallops. This concentration (10 ppm) is rarely observed under naturally dispersed oil slicks and certainly rarely occurs at the seabed. This indicates that the probability of tainting in sea scallops due to a short-term condensate blowout in offshore waters must be low. The same is true for toxic effects. A long-term blowout might increase the risk of tainting.

RÉSUMÉ

Une éruption de composés liquides (condensat) du gaz liquide naturel, survenue en 1984 au large de l'île de Sable, a suscité des inquiétudes à cause de la possibilité d'altération du goût (développement d'un arrière-goût dans le tissu comestible de certaines espèces commerciales) et d'effets toxiques, selon les conditions prévalantes, si d'autres éruptions de condensat surviennent dans le futur. La présente étude portait sur l'altération du goût et la toxicité des pétoncles géants (Placopecten magellanicus) exposés à la fraction hydrosoluble (FH) du condensat de la plate-forme Scotian. Présentment, on fait la récolte des pétoncles près de champs gaziers possibles au large de l'île de Sable.

Les objectifs de l'étude étaient d'évaluer les propriétés chimiques du condensat de la plate-forme Scotian, de déterminer la possibilité d'altération du goût dans les pétoncles exposés à la FH du condensat de la plate-forme Scotian.

On a recueilli des pétoncles dont la hauteur de la coquille dépassait 8 cm dans la baie de Fundy. Ils ont été conservés dans des installations de conservation à l'extérieur d'Halifax. Des expériences ont été effectuées à l'Université Dalhousie et à l'Institut océanographique de Bedford. La fraction hydrosoluble a été préparée dans un contenant d'acier inoxydable de 1,100 L, avec un rapport de mélange de 1:299 (condensat: eau de mer) immédiatement avant son utilisation pour les expositions d'altération du goût et de la toxicité. Des essais de toxicité ont été effectués dans des contenants immobiles de 20 L à l'aide de diverses dilutions de FH. Les observations portaient entre autres sur les taux de mortalité différés moins de cinq jours après les expositions. Pour l'évaluation de l'altération du goût, on exposait les pétoncles à un écoulement continu de FH et d'eau de mer utilisée comme diluant, ce qui permettait de maintenir des concentrations relativement stables d'hydrocarbures. Celles-ci étaient surveillées fréquemment à l'aide d'un fluoromètre. Les résultats des analyses ont été étalonnés par chromatographie gazeuse. Les expositions d'altération du goût duraient de 3 à 96 h. On a également étudié la dépuration chez des pétoncles exposés à la FH de 24 à 96 h. La méthode de micro-extraction Murray a été utilisée pour l'extraction des hydrocarbures des échantillons. On a analysé le condensat, l'eau d'exposition et les muscles adducteurs des pétoncles par chromatographie gazeuse et par chromatographie gazeuseéspectrométrie de masse. L'altération du goût a été évaluée par la méthode du jury de dégustation, et elle était basée sur les essais triangulaires avec des échantillons témoins.

Les principaux constituants du condensat de la plate-forme Scotian étaient des hydrocarbures aromatiques, dont les plus abondants étaient le benzène, le toluène et des xylènes. On observait également des concentrations moyennes de naphthalène ainsi que de ses dérivés méthyl et diméthyl. La concentration des hydrocarbures de la FH était comprise entre 42 et 56 ppm. La FH comportait principalement des composés aromatiques à faible poids moléculaire. On observait moins de n-alcanes dans la FH que dans le condensat dont celle-ci provenait.

La CL_{50}-96 h pour les pétoncles géants exposés à la FH de condensat était de 17,4 ppm (concentration initiale mesurée). Quand les cas de mortalité différée étaient inclus, la CF_{50}-96 h était de 12,5 ppm.

L'altération du goût était évidente chez des pétoncles exposés pendant trois heures à 2,1-4,8 ppm de FH. L'altération était également évidente chez des pétoncles exposés pendant 24 heures à 3,8 ppm, mais à 0,8 ppm, aucune altération n'était décelée. Le goût des pétoncles exposés à 0,8 ppm pendant 96 h était altéré de façon significative, ce qui n'était pas le cas de ceux qui étaient exposés à 0,6 ppm. Les pétoncles dont le goût était altéré de façon significative après une exposition de 24 h à une FH d'une concentration de 2,7 ppm étaient exempts d'altération du goût après 24 heures dans l'eau de mer. Par ailleurs, les pétoncles dont le goût était altéré après une exposition de 96 h à une concentration de FH de 0,9 ppm ne retrouvaient leur goût initial qu'après avoir passé entre 96 et 264 h dans l'eau de mer propre.

Les pétoncles exposés pendant 24 h à une concentration de FH supérieure ou égale à 2.7 ppm présentaient une absorption importante d'hydrocarbures dans le muscle adducteur. Ceux dont le muscle adducteur présentait une concentration supérieure à 15,6 % avaient un goût significativement altéré. Les pétoncles exposés à moins de 1 ppm de FH ne présentaient pas d'absorption significative d'hydrocarbures et leur goût n'était pas altéré. L'analyse indiquait une perte rapide des hydrocarbures dans les muscles adducteurs à goût altéré étaient le toluène et les xylènes. Le naphthalène, le méthyl- et le diméthylnaphthalène s'accumulaient également dans le muscle adducteur.

Les résultats analytiques n'indiquaient que très peu d'altération du condensat avant son utilisation dans les essais d'exposition pour étudier l'altération du goût et la toxicité. Le condensat avait une solubilité relativement élevée par rapport aux divers pétroles bruts. Toutefois, il était aussi très volatile, 90 % de celui-ci s'évaporant en moins de 24 h au cours des essais d'exposition. Il y avait une perte minime de condensat au cours des expositions à cause de l'apport continu de FH, qui permettait de maintenir des concentrations relativement stables d'hydrocarbures. Au cours de l'analyse des FH, les taux de récupération des hydrocarbures étaient relativement faibles, mais la répétabilité était bonne, ce qui indique un niveau de confiance élevé pour les données d'exposition dans l'eau. Les taux de récupération des hydrocarbures dans les muscles adducteurs étaient très faibles, de sorte que les données des taux d'hydrocarbures dans le muscle adducteur peuvent être considérées comme semi-quantitatives.

La FH du condensat de la plate-forme Scotian était semblable aux FH de divers pétroles bruts, ce qui indique que la nature de la fraction de pétrole réellement dissoute est relativement normale. Des essais de toxicité ont indiqué que les pétoncles étaient moins sensibles aux composés toxiques du condensat que diverses espèces de poisson qui ont été testées. Toutefois, les pétoncles étaient au moins deux fois plus sensibles que les oursins.

Le seuil d'altération du goût pour le pétoncle au cours des expositions de 3-96 h à des FH de condensat est de 0,8 ppm. Une augmentation du temps d'exposition à un niveau d'exposition donné tendait à augmenter le degré d'altération, et plus particulièrement près de la valeur seuil. La valeur seuil d'altération pour la FH du condensat de la plate-forme Scotian est semblable à celle de la FH du pétrole brut ninien (0,5 ppm). Pour les pétoncles exposés à de faibles concentrations de FH (moins de 1 ppm), le taux d'accumulation des hydrocarbures dans le muscle adducteur n'était que de 1,6-1,8 fois le taux d'exposition. Aux taux d'exposition élevés (2,7-3,8 ppm), l'accumulatin des hydrocarbures était de 5,9-12,9 fois le taux d'exposition et le goût de ces pétoncles était significativement altéré. Même si le toluène et les xylènes n'étaient pas les composés les plus significatifs des

muscles adducteurs exposés, ils n'étaient accumulés qu'à une concentration reflétant leur proportion dans la FH, ce qui indique l'absence d'absorption ou de rétention sélective de ces composés aromatiques légers. Par ailleurs, il y avait rétention sélective de benzène alkylé, du naphthalène, ainsi que des naphthalènes alkylés.

Le toluène et les xylènes jouaient un rôle dans l'altération du goût des pétoncles géants. De plus fortes concentrations de benzène alkylé, de naphthalène et de naphthalènes alkylés peuvent également jouer un rôle dans l'altération du goût; toutefois, ils ne modifient pas le goût quand ils sont présents en faibles concentrations dans le muscle adducteur. La dépuration rapide des muscles adducteurs de pétoncles altérés était problablement en rapport la réduction rapide la concentration des composés aromatiques légers. Après 24 h de dépuration, il ne restait que 8 % des hydrocarbures accumulés.

Les données de rapport de mélange indiquent qu'une FH réelle ne comprend pas plus de 2-8 % du volume du condensat entier, selon le rapport des mélanges. Avec un seuil d'altération du goût de 0,8 ppm, la concentration du condensat entier dans la colonne d'eau doit êntre maintenue à au moins 10 ppm pour causer une altération du goût chez les pétoncles géants. Cette concentration (10 ppm) est rarement observée dans des nappes d'hydrocarbures naturellement dispersées et on peut dire qu'elle est certainement rare au fond de la mer. Ceci indique que la probabilité d'altération du goût des pétoncles géants due à une courte éruption de condensats dans les eaux du large est tres faible. Il en va de même pour les effets toxiques. Une longue éruption pourrait augmenter le risque d'altération du goût.

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1.0 INTRODUCTION

In 1984, a blowout at the Shell Uniacke G-72 well off Sable Island resulted in contamination of the marine environment with natural gas liquids (commonly referred to as condensate) (Carter et al., 1985). There was no evidence of tainting of fish as a result of the blowout (Martec Limited, 1984), but there was some indication of uptake of hydrocarbons by cod and haddock (Zitko et al., 1984). Zitko et al. suggested that the potential of condensate to contaminate fish is higher than anticipated previously. Because of these concerns and the potential for development of the Scotian Shelf gas fields, further work on the tainting and toxic potential of Scotian Shelf condensate was deemed necessary.

The purpose of this project was to examine exposure time and concentration of the water-soluble fraction (WSF) of Scotian Shelf condensate as factors in the development and persistence of tainting in sea scallops (Placopecten magellanicus) and to examine lethal toxicity in scallops exposed to condensate WSF.

Tainting is the development of an atypical flavour or odour in the edible tissues of finfish and shellfish caused by natural spoilage or by the assimilation of contaminants. Tainting as a result of exposure to petroleum hydrocarbons has been documented frequently (Mackie et al., 1972; Nitta, 1972; Shenton, 1973; Motohiro and Inoue, 1973; Connell, 1974; Kerkhoff, 1974; Scarratt, 1980). Concern for fisheries resources which may be exposed to oil from spills or from oil-based drilling mud has led to several recent studies in which tainting thresholds were examined and individual hydrocarbons in contaminated tissues were determined (McGill et al., 1987, Ernst et al., 1988 a,b). The principal components of crude and refined oil which have been implicated in tainting include the phenols, dibenzothiophenes, naphthenic acids, mercaptans, methylated naphthalenes, toluene, and other alkylbenzenes (GESAMP, 1977; Motohiro, 1983). The aromatic fraction of Scotian Shelf

condensate is mainly composed of benzene, toluene, xylenes, methylbenzenes, naphthalene, and methyl and dimethyl derivatives of naphthalene (Martec Limited, 1984; Zitko et al., 1984). Scotian Shelf condensate thus appears to have a potential for tainting.

The toxicity of condensate has been examined in several species, including salmon (Zitko et al., 1984), trout and sea urchins (Hutcheson et al., 1983), mussels (E.P.S., 1983), Daphnia (Bobra et al., 1983), and mummichogs (Mahon et al., 1987). Since none of these organisms is likely to occur at significant densities near the location of a natural gas blowout on the Scotian Shelf, toxicity testing with an appropriate organism was still required.

The sea scallop (Placopecten magellanicus) was selected as the test organism for the tainting and toxicity experiments because it is presently harvested near potential gas fields on the Scotian Shelf and there is a comparative data base for tainting of scallops from Georges Bank (Ernst et al., 1988b).

The three main objectives of the study were:

1. to assess the chemical properties of the condensate used in the study;

2. to determine in sea scallops the acute toxicity of Scotian Shelf condensate;

3. to determine the potential for condensate tainting in sea scallops.

The report is organized in the usual format of materials and methods, results, and discussion. A series of appendices provides details on analytical recovery and precision, examination of the exposure system, calibration of the various analytical methods, and the individual analytical results.

2.0 MATERIALS AND METHODS

2.1 Scallop Acquisition and Holding

Over 4,000 sea scallops were collected from the lower Bay of Fundy in early February and early March, 1988 by a scallop dragger from Digby, Nova Scotia. Most of the scallops had a shell height exceeding 8 cm. The scallops were transported by truck to a holding facility. They were not out of the water for more than 3 hours.

The scallops were kept at the National Research Council (NRC) Facility at Sandy Cove, Nova Scotia. The open ocean water supply was drawn from 6 m water depth and passed through a coarse filter which allowed passage of plankton for scallop feeding. Scallops were held in a 4 x 7 x 0.7 m lined concrete tank, covered with a plastic tarpaulin. Water flow into the tank ranged from 100 to 120 L/minute. Aeration was provided. The water temperature increased from 1-2 °C in February to 3-4°C in May when the experiments were completed. The scallops were checked regularly and dead individuals were removed. The cumulative mortality rate over three months was 5%.

Scallops used for the toxicity tests were tranferred to holding tanks at the Bedford Institute of Oceanography (BIO) where they were acclimated to 10°C seawater. Any scallops which appeared unhealthy were removed from the tanks.

Four days prior to a proposed exposure for taint evaluation, the required number of scallops were transferred from the NRC facility to tanks at Dalhousie University. Seawater at Dalhousie was drawn from the Northwest Arm of Halifax Harbour and passed through four pressure sand filters.

2.2 Test Media

2.2.1 Condensate

Venture condensate (two batches totalling 140 L) was provided by Environment Canada for this study. The two batches were blended and this mixture was used to prepare WSFs for all toxicity and tainting exposures.

2.2.2 Water-Soluble Fraction

All water-soluble fractions (WSF) used in the exposure trials were prepared in a 1,100-L stainless steel mixing vessel equipped with a powerful mechanical stirrer, a bottom drain, and a cooling-water system (Figure 2.1). The stirrer consisted of a three-blade propeller just above the bottom of the vessel, attached to a vertical shaft, and driven by a 2.2 horsepower electric motor mounted on top of the vessel. The action of the mixing was such that a vortex was produced.

Condensate and seawater, in a ratio of 1:299 (volume-to-volume) (see Appendix A), were stirred for 24 h and allowed to settle for an additional 48 h. The WSF was kept cool by circulating cold water through the outer jacket of the mixing vessel. WSF was prepared immediately prior to its use in exposure experiments.

WSF was stored in the closed mixing vessel to minimize the loss of hydrocarbons to the atmosphere through evaporation. WSF was transferred to 20 L buckets and transported to BIO on a daily basis as required for the toxicity tests. WSF used in exposures for taint evaluation was pumped directly out of the mixing vessel from the bottom drain and into a small head tank for controlled flow into the exposure tanks.

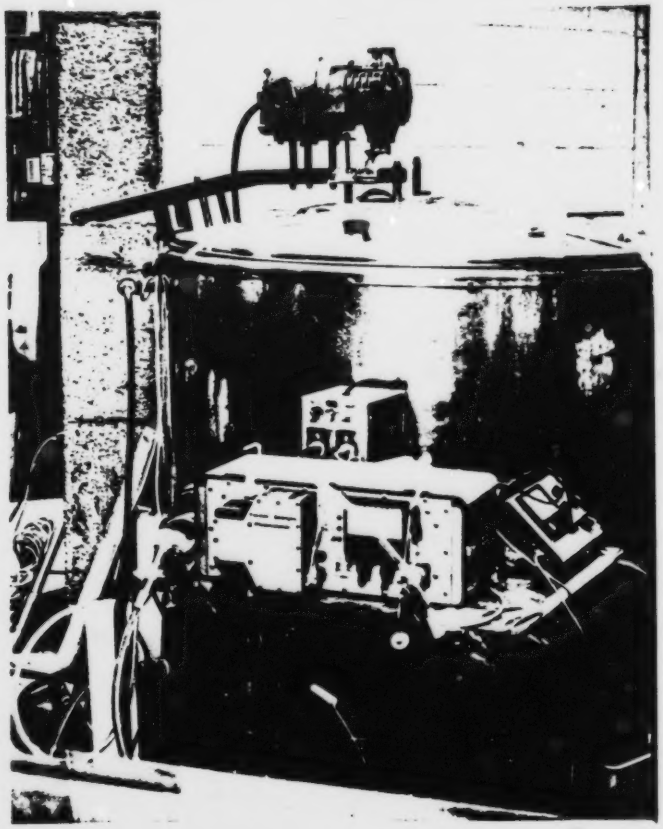


Figure 2.1. WSF mixing vessel with fluorometer in the foreground.

2.3 Exposure of Scallops to Water-Soluble Fraction

2.3.1 Toxicity Tests

Toxicity tests were conducted by Seakem Oceanography Limited. The scallops were exposed to the condensate WSF in static 20 L test containers which were supplied with minimal aeration and fitted with a plastic lid. Seven scallops were used in each test container during the range-finding trial (48 h) and 10 scallops were used in each container during the 24 h and 96 h trials. All test containers were maintained at 10°C in a regulated water bath. During the 48 h and 96 h exposures, the scallops were transferred daily to test containers with freshly prepared WSF mixtures. Each concentration was run in duplicate and the water temperature, pH, and dissolved oxygen were measured daily. The hydrocarbon concentration in each container was monitored every 24 h (see Section 2.4).

A five-day recovery period followed each exposure. It was expected that some of the scallops placed in clean running seawater after exposure to WSF would exhibit delayed mortality and some scallops would recover from narcotization. Tentacle extension (one of the primary indicators of recovery) was recorded during the five-day recovery periods. A scallop was considered to be dead when it did not respond to tactile stimulation of the mantle and tentacles.

Three tests were conducted to examine the toxicity of condensate WSF. The first was a 48 h range-finder with a wide concentration range of 100% to 10% WSF. This was followed by 24 h and 96 h LC50 determinations.

The mortality data were analyzed using the 1982 version of Charles E. Stephan's LC50 analysis program. The results of the computer analysis were checked graphically.

2.3.2 Taint Evaluation

2.3.2.1 Exposure System

Figure 2.2 is a schematic representation of the exposure system used for the taint evaluation trials. The exposure experiments were conducted in 1 x 1 x 0.5 m (355 L) fibreglass tanks equipped with bottom corner drains (Figure 2.3). All tanks were covered with 6-mm clear plexiglass tops with a 2 cm strip of foam tape where the plexiglass contacted the tank. The drain system was designed so that the overflow water came from the bottom of the tank to ensure good mixing of the exposure water. The exposure and control tanks were plumbed separately to ensure no possibility of hydrocarbon contamination through the drain systems. Depuration was carried out in tanks that were identical to the exposure tanks except that they were not covered with plexiglass tops. Exposures were run using a flow-through system based on a 1 L/minute requirement of well-oxygenated water.

The diluent seawater was bubbled with pure oxygen to a super-saturated condition in a large (400 L) constant head tank (Figure 2.4). The oxygen level in the exposure tanks was monitored regularly with a YSI Dissolved Oxygen Meter and the flow of oxygen to the head tank adjusted to maintain an oxygen level of between 70 and 100% saturation in the exposure tanks.

The flow rate of diluent seawater to each tank was controlled by clamping the individual lines from the head tank to the exposure tank. The WSF flow rates were similarly controlled. WSF was pumped from the bottom drain of the mixing vessel to a small (5 L) covered constant head tank (Figure 2.5). The overflow from this head tank was directed back into the mixing vessel to conserve WSF.

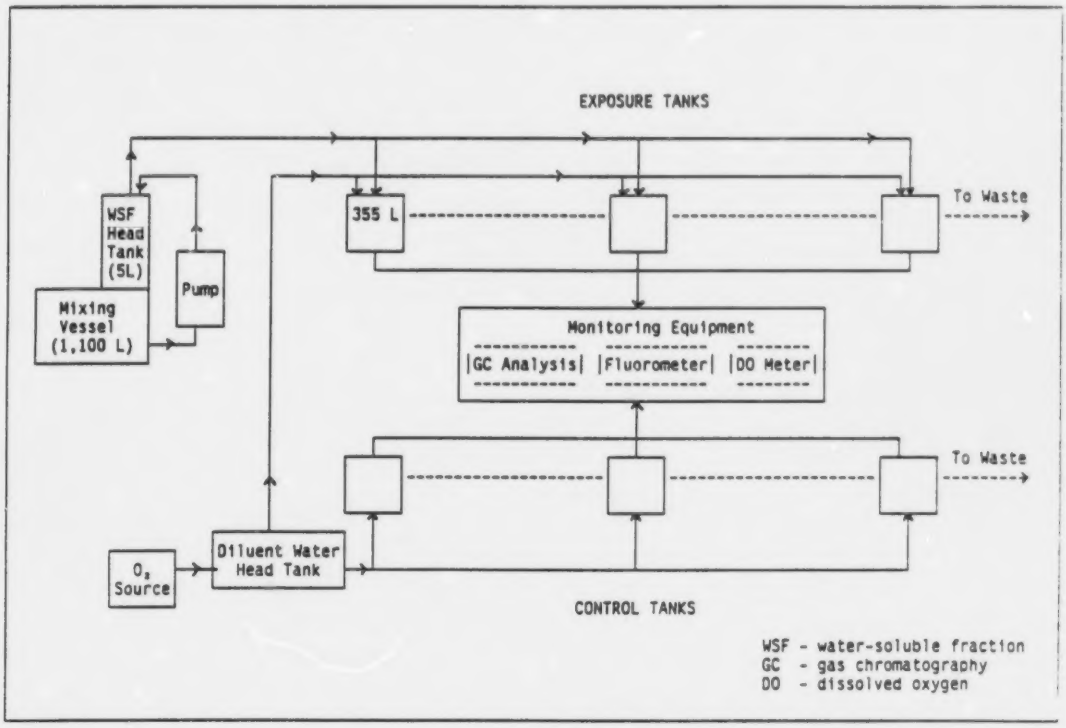


Figure 2.2. Test system configuration for tainting evaluation.

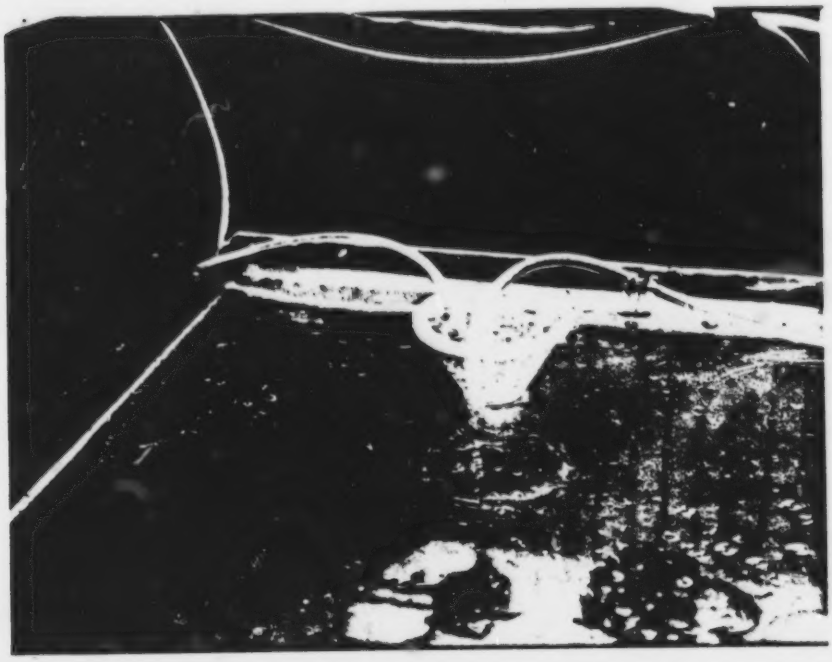


Figure 2.3. Exposure tank fitted with WSF and diluent seawater lines.

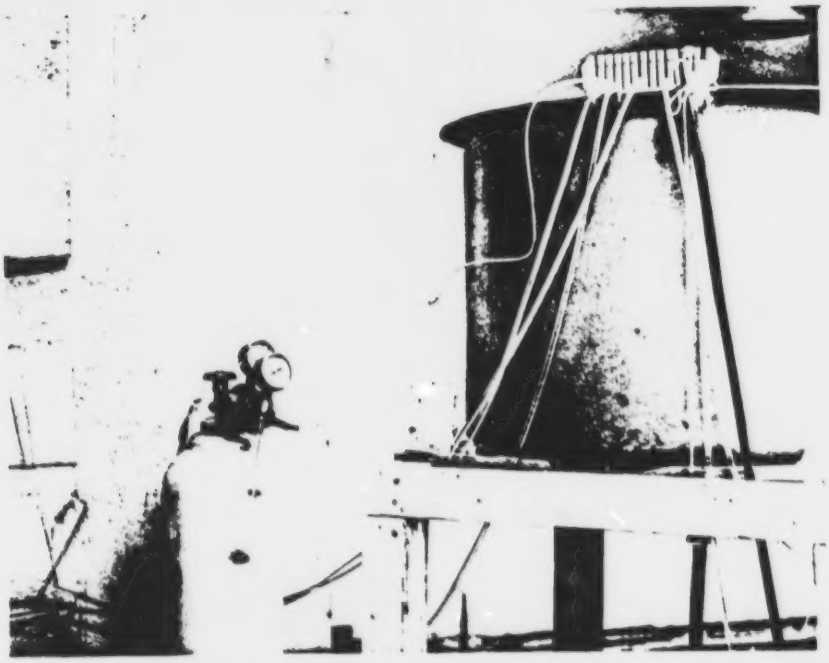


Figure 2.4. Diluent seawater tank.

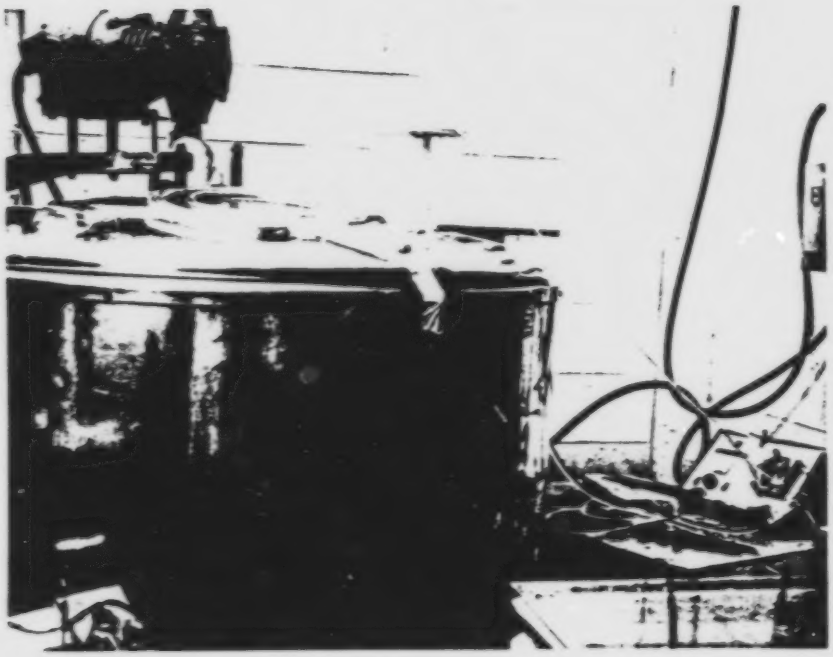


Figure 2.5. WSF head tank on top of mixing vessel and WSF pump to the right.

The WSF and diluent seawater streams emptied together into a covered funnel fitted on a 14 cm long plexiglass tube attached to the bottom of the tank cover. The streams mixed as they flowed down the tube and into the exposure water (Figure 2.3). The hydrocarbon concentration was monitored during two mock trials prior to exposures with scallops to determine the stability of this exposure system (see Appendix B). Control tanks received only oxygenated diluent water. Scallops were introduced into the exposure water by lifting up one corner of the plexiglass top and quickly sliding them into the tank taking care that they were evenly distributed on the bottom.

The hydrocarbon concentration in each tank was monitored regularly using a Turner Designs Model 10 fluorometer fitted with a short wavelength (254 nm) oil kit (Figure 2.1). Water samples for GC analysis were taken at the beginning and end of the shorter exposures ($\leq$24 h) and also at mid-time during the longer exposures (96 h). These samples were used to calibrate the fluorometric data (Appendices C, D, and E). Temperature and dissolved oxygen were also monitored on a regular basis and the behaviour of the scallops was noted.

After exposure to the WSF, the scallops were removed from the tank and rinsed in clean seawater, then immediately transferred to the Canadian Institute of Fisheries Technology (CIFT) where they were prepared for either tissue analysis or taste panel evaluation.

2.3.2.2 Experimental Design

A series of trials was proposed to examine the variables of exposure time and hydrocarbon concentration which can affect the development of a taint. A range of hydrocarbon concentrations for 3 exposure periods comprised a total of 9 exposure trials. The length of time required for tainted scallops to depurate was also studied. Scallops were exposed to hydrocarbon concentrations known to induce a taint in 24 h and 96 h.

After exposure, the scallops were transferred to uncontaminated, clean seawater for the depuration phase.

The actual exposure times, depuration times, and concentrations selected for each trial were determined after consideration of the results of previous trials. Table 2.1 shows the conditions and order in which the trials were conducted.

The first 24 h exposure was treated as a range-finding trial designed to determine the general range of hydrocarbon concentrations in the water which can taint scallop adductor muscle. The next trial was a 24 h exposure followed by depuration. For the 96 h (long-term) exposure, the two hydrocarbon concentrations which did not induce a taint after 24 h (~0.5 and 0.8 ppm) were repeated. A higher exposure level was also included. In the 3 h trials, two of the concentrations selected were in the range which was known to induce a taint in scallops after 24 h. The third exposure level in this trial was higher. The last trial was designed to examine the time required for depuration after long-term low-level exposure.

2.4 Hydrocarbon Analysis of Samples from the Toxicity Tests

Chemical analysis of the exposure water used in the toxicity tests was performed by extracting one litre samples with freon 113 and comparing the fluorescence of the extract with a standard curve derived from the condensate. The maximum condensate fluorescence occurred at an excitation wavelength of 300 nm and an emission wavelength of 330 nm. The results obtained using this method were not directly comparable to the results in the tainting portion of the project as different techniques were used. An intercalibration was conducted (Appendix F). The results of the toxicity analysis presented in Section 3 have been adjusted for direct comparison with the analysis of the original stock WSF used in the tainting trials.

Table 2.1. Summary of the exposure conditions for taint evaluation.

Exposure Time (h)	Average Hydrocarbon Concentration (ppm) in Exposure Water	Depuration Time (h)
24 h	0.45[1]; 0.78[1]; 3.79[1]	---
24 h (depuration)	2.72; 2.68; 2.57	0[1]; 24[1]; 96[1]; 216[1]
96 h	0.55; 0.81; 1.41	---
3 h	2.10; 3.55; 4.76	---
96 h (depuration)	0.98; 0.89; 0.90	0; 24; 96; 264[1]

[1]adductor muscle also analyzed by gas chromatography.

2.5 Hydrocarbon Analysis of Samples from the Taint Evaluation

2.5.1 Preparation of Samples of Water-Soluble Fraction

Water samples for hydrocarbon analysis were collected by siphoning exposure water from the tanks into either amber or foil-wrapped glass bottles. The bottles were rinsed with the sample water, then were filled to capacity. They were covered with aluminum foil before the top was put on. When possible, the sample was analyzed immediately; otherwise it was held at 5°C until analyzed. The hydrocarbons in the WSF were extracted according to the Murray microextraction procedure (1979 and 1984), described below.

An extraction flask of capacity slightly over 1 L was constructed with a side arm and a capillary tube at the top-centre of the flask. This extraction flask was the same as that described by Murray (1979). The WSF (980 mL) was placed in the flask and the mixture was shaken with hexane (1 mL) for 2 minutes. The flask was stoppered and the layers were allowed to settle for a minimum of 15 minutes in a domestic refrigerator (~5°C) to permit the hexane droplets dispersed throughout the aqueous phase to collect on the surface. The hexane layer was directed to the centre capillary tube by adding distilled water (70-80 mL) through the side arm, while tilting the flask at an angle of about 45°. One µL of the hexane layer from the capillary tube was taken into a 10 µL Hamilton GC syringe containing 0.2 µL of clean hexane, followed by 1 µL of a hexane solution of n-heneicosane ($n\text{-}C_{21}$; 0.561 µg/µL). Both were injected simultaneously into the GC (see Section 2.5.4). Quantitative analyses in all cases were based on the use of $n\text{-}C_{21}$ as the internal standard.

The areas of the peaks were integrated and concentrations were calculated from the ratio to the internal standard. The hexane used in

the extraction contained very low levels of cyclohexane, methylhexane, isooctane and heptane. Purification of the hexane by gentle fractional distillation did not remove these impurities. The areas of the peaks due to hexane and its impurities were therefore subtracted in the calculation. These experimentally determined concentrations were corrected for the recovery efficiency of the microextraction technique.

The recovery efficiency of the microextraction technique and the gas-liquid chromatography (GLC) system response correction factors for three concentrations of 27 hydrocarbons were investigated. For each concentration, the whole procedure was triplicated and duplicate GC analysis was performed with each hexane extract (Appendix G). The final correction factors (calculated by multiplying the GLC system response correction factor by the recovery efficiency correction factor) ranged from 1.24 to 11.90 (see Table G.5). The low molecular weight hydrocarbons such as heptane, isooctane, octane, and benzene needed a higher correction factor because of their relatively lower recovery from the WSF.

2.5.2 Preparation of Samples of Condensate

Condensate (~20 mg) was dissolved in distilled hexane (1-5 mL) and 1 µL of this solution was analyzed by capillary gas-liquid chromatography (see Section 2.5.4).

2.5.3 Preparation of the Scallop Adductor Muscle

The hydrocarbons in the scallop adductor muscle were extracted by the steam distillation procedure of Ackman and Noble (1972).

The distillation apparatus consisted of a 500-mL round-bottomed flask, a Barrett-type distilling receiver, and a gooseneck tube attached to a graduated collection flask, with the addition of an overflow to

return excess solvent to the flask, and a water condenser. The water condenser was connected to the graduated collection flask of the Barrett-type distilling receiver. A fresh, pre-rinsed (with methylene chloride), coarse, glass wool plug was placed at the inside joint of the gooseneck tube and the 500-mL round-bottomed flask prior to each distillation of hydrocarbons from the adductor muscle. This technique prevented any foam from being transferred directly into the graduated collection flask. Bumping of water was minimized with a few glass beads. The whole apparatus was rinsed with distilled water and acetone and dried shortly before use.

Distilled water (270 mL) was placed in the 500-mL flask and was heated to boiling using an electric heating mantle. The condensate was collected into the graduated collection flask. The distillation was terminated after 20 mL of condensate were collected. The flask of the distillation apparatus was cooled in ice and the inside of the condenser was rinsed with 2 mL of methylene chloride. The water condensate and methylene chloride were discarded. Minced adductor muscle (50 g) from 15 to 25 scallops was added to the remaining water (250 mL) in the distillation flask. The distillation was continued until 20 mL of condensate were collected. The flask of the distillation apparatus was cooled in ice, the condenser was rinsed with methylene chloride (2 mL) into the collected condensate, and the whole was drained into a separatory funnel and mixed. The methylene chloride and the aqueous layers were allowed to separate. The methylene chloride extract was collected, dried over sodium sulphate, and an aliquot (1 µL) was analyzed by GC (see Section 2.5.4), and by GC/MS (see Section 2.5.5). Quantitation of the hydrocarbons was based on the use of the n-C_{21} internal standard as described previously (Section 2.5.1). These experimentally determined concentrations were corrected for the recovery efficiency of the steam distillation procedure.

Similarly, hydrocarbons from the scallop adductor muscle from the control trials were isolated by the steam distillation procedure and analyzed by GC. The recovery efficiency of the steam distillation procedure was investigated for nine hydrocarbons. The final correction factors (calculated by multiplying the GLC system correction factor by the recovery efficiency factor) ranged from 4.7 to 19.51 (Appendix H).

2.5.4 Gas-Liquid Chromatography

The hydrocarbon analyses were conducted with a Perkin-Elmer 8420 capillary gas chromatograph equipped with a flame ionization detector (FID) and a split injection system. The chromatography was conducted on a DB-1 (60 m x 0.25 mm I.D., 0.25 micron) flexible, fused-silica capillary column (polymethysiloxane phase, chemically cross-linked, and surface bonded; J and W Scientific, Inc., Folson, California).

Conditions and temperatures were as follows: FID, 280°C; injector, 280°C. Column temperature was programmed as follows: initial temperature, 45°C; held for 15 minutes, then programmed at 13°C per minute up to 280°C and held for 30 minutes. The carrier gas (helium) pressure was 20 pounds per square inch gauge (psig) and the hydrogen and air pressures of the FID were 13 and 21 psig, respectively.

The concentration of hydrocarbons in the samples was calculated with respect to the internal standard, n-heneicosane (C_{21}). This standard was ideal as it is not readily volatile and the evaporation losses are minimal. It eluted on the column without interfering with any of the petrogenic hydrocarbons found in the WSF.

The peak areas were recorded and integrated on a Perkin-Elmer GP-100 graphics printer integrator (Perkin-Elmer, Norwalk, Connecticut). The areas of all the peaks, excluding those contributed by hexane and the blank run, were summed and the concentrations were calculated from the ratios to the internal standard.

In calculating the total hydrocarbon concentrations, the components that eluted ahead of the solvent peak were not taken into account. These are most likely aliphatics below n-C_8, such as pentane, butane and their isomers. These are quite volatile and are not usually present in dilute solutions of WSFs and, if present, would evaporate off during the actual exposure period.

2.5.5 Gas-Liquid Chromatography/Mass Spectrometry (GC/MS)

The component hydrocarbons in the water-soluble fractions and in the scallop adductor muscle were identified by using a Finnigan MAT 700 Ion Trap Detector (ITD) form of gas-liquid chromatography/mass spectrometry. The Finnigan MAT 700 Ion Trap Detector system is controlled by an IBM Personal Computer XT (PC-XT). The microcomputer also handles all data storage and processing. The identifications of hydrocarbons were facilitated by the use of the 43,000 spectra library in the hard disk of the PC XT.

The ITD was interfaced to a Perkin Elmer 990 gas chromatograph. The chromatography was executed on a DB-1 column (28 m x 0.25 m) fused silica capillary column. The column temperature was programmed as follows: initial temperature 45°C and held for 16 minutes, then programmed at a rate of 12°C per minute up to 280°C and held for 24 minutes. The helium carrier gas pressure was 10 psig.

2.6 Taste Panel - Sensory Evaluation of Taint

2.6.1 Taste Panelists

The taste panelists were staff and students of the Canadian Institute of Fisheries Technology (CIFT) and had experience in sample tasting. Most of the members had participated in at least seven previous taste panel studies conducted by CIFT. Several of these studies involved petroleum hydrocarbons. Each panel had 13 to 15 panelists.

2.6.2 Preparation of the Scallop Adductor Muscle

After each exposure, the scallops were rinsed in clean seawater and immediately transferred to CIFT where they were shucked. The adductor muscles were rinsed in fresh water, then were placed in polyethylene bags and stored in a -35°C cold room.

The taste of scallop adductor muscle exposed to condensate WSF was evaluated with respect to a control group. The control group of scallops was held in tanks under the same conditions as the exposed scallops, except that the seawater was not spiked with WSF. The control scallops were shucked and stored exactly like those of the corresponding exposed group. Several scallop adductor muscles were tested for paralytic shellfish poison (PSP) prior to the first taste panel evaluation. The toxin levels were determined to be negligible.

Scallops (exposed and control scallops being kept separate) used for sensory evaluation were minced in a Cuisinart food processor for 10 to 15 s to ensure a homogeneous sample. A sample of 10 to 15 g was formed into a patty, placed in a covered glass petri dish, and cooked in a microwave oven for 45 s before presentation to the taste panelists.

2.6.3 Sensory Evaluation of Samples by Triangle Test

The triangle test was used to detect tainting as recommended by Tidmarsh *et al.* (1985) and GESAMP (1983). The triangle test involved presenting the panelists with three samples; two were identical and one was different (two controls + one exposed or two exposed + one control). Each panelist was asked to select the odd sample. If there were 16 panelists, eight were presented with two controls and one exposed sample and eight received two exposed samples and one control. The petri dishes were placed on a tray in different triangular formats. The score sheet that was used is shown in Figure 2.6. In addition to determining the odd

TRIANGLE TEST - SCALLOPS

NAME _____

DATE _____

One of these three samples is different from the other two.

1. Test the samples in order indicated and identify the odd sample.

Code	Check Odd Sample
____	_____
____	_____
____	_____
____	_____

2. Indicate the degree of difference between the duplicate samples and the odd sample.

Slight _____

Moderate _____

Much _____

Extreme _____

3. Acceptability:

Odd sample more acceptable _____

Duplicates more acceptable _____

4. Comments:

Figure 2.6. Taste panel questionnaire.

sample, the panelists were asked if they preferred the odd or the duplicate sample.

The sensory evaluations were conducted at the taste-testing facilities of CIFT. The testing area had good ventilation for removal of cooking odours to prevent bias in the results. The panelists were in individual booths and therefore were not influenced by other panelists. The testing area was illuminated with fluorescent red light to avoid bias induced by sample appearance. Each panelist was given a glass of water and a glass of a mixture of equal parts of water and Diet 7-UP. The panelists were asked to rinse the mouth by taking a sip of water or the mixture of liquids between the samples tested.

2.6.4 Evaluation of Results

The taste panel results were evaluated statistically as described by Larmond (1977). In the triangle test there is a 1/3 chance of obtaining correct results. Appendix I shows the minimum number of correct responses for various levels of significance for a wide range of panelists. As can be seen from this table, as the number of panelists increases, the proportion of correct responses to obtain a significant difference decreases.

In those trials in which a significant difference was observed, the data were re-examined. Of those who correctly picked the odd sample, the number who preferred the control sample was noted.

3.0 RESULTS

3.1 Test Media

3.1.1 Condensate

Figure 3.1 is a chromatogram of the blended condensate used for the toxicity and tainting exposure work. The major components in the condensate were aromatic hydrocarbons, whereas the aliphatics were present in low concentrations. The GC profile shows the presence of high levels of benzene, toluene and xylenes. Among the other aromatics were complex patterns of tri- and tetramethylbenzenes. The main polymethyl benzene appears to be 1,2,4-trimethylbenzene. The condensate showed moderate levels of polynuclear aromatics (PNAH) composed mainly of naphthalene and its methyl and dimethyl derivatives. Higher naphthalenes and other PNAH were also detected, but at low levels, eluting in the n-C_{18} to n-C_{21} region of the gas chromatogram.

In terms of alkanes, the condensate showed hydrocarbons from n-C_7 to n-C_{26} with those from n-C_8 to n-C_{13} present in approximately equal concentrations. The concentrations gradually decreased from n-C_{14} to n-C_{26}. Apart from the aromatics and alkanes, the condensate had a high level of methylcyclohexane, almost equal to that of toluene.

3.1.2 Seawater

The uncontaminated seawater used in this study was examined a number of times for background levels of hydrocarbons by the Murray microextraction technique and gas chromatography. The seawater samples had no detectable levels (<0.001 ppm) of petrogenic hydrocarbons. The peaks observed in the chromatogram were those contributed by the extracting solvent, hexane and its impurities.

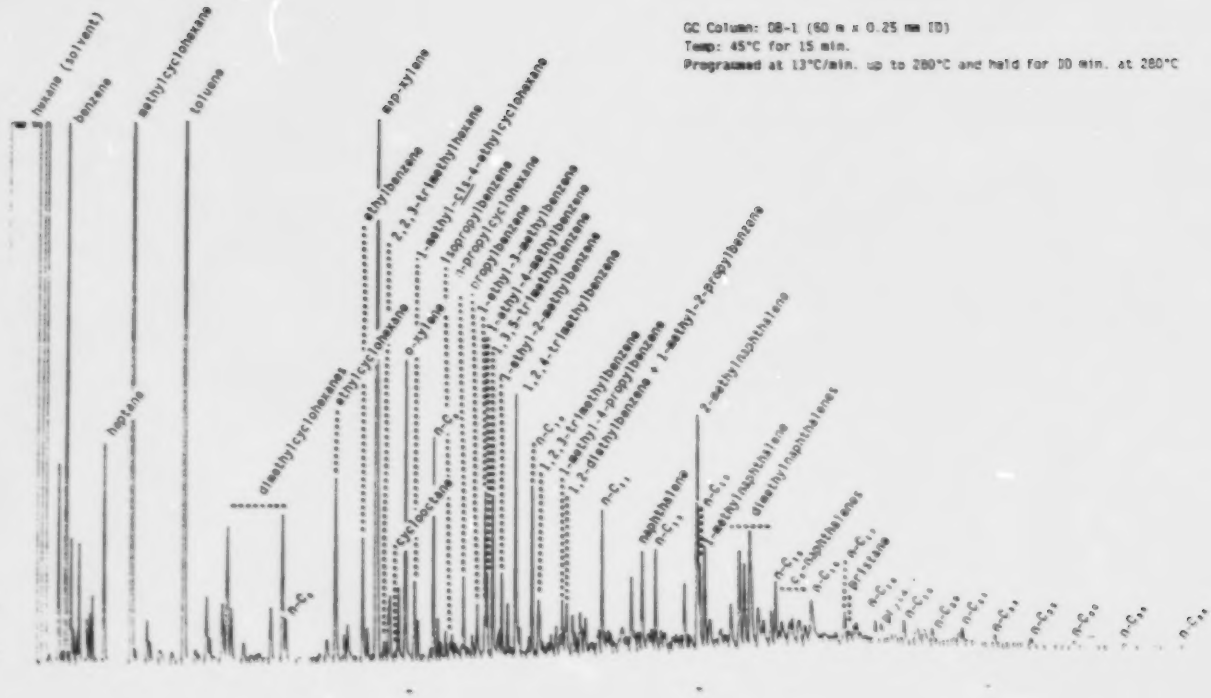


Figure 3.1. GLC hydrocarbon profile of the blended condensate.

3.1.3 Water-Soluble Fraction

Several stock WSF batches were prepared for the various exposure trials. The total hydrocarbon concentration of the stock WSF ranged from 42 to 56 ppm.

In one of the preparations, the hydrocarbons were characterized in detail by GC and GC/MS. Figure 3.2 is a chromatogram of a typical WSF prepared in the large mixing vessel. Table 3.1 lists the corresponding hydrocarbon composition and the major fragments in their mass-spectra. The hydrocarbon profile of the WSF was somewhat different from the original condensate (Figure 3.1). The major difference was in the n-alkanes; these were almost absent in the WSF, except for trace levels of n-C_7 to n-C_{12}. The WSF, like the parent condensate, was dominated by low molecular weight aromatic hydrocarbons. Toluene, benzene, and xylenes were the major hydrocarbons in the WSF. The other dominant hydrocarbons were methylcyclohexane, ethylbenzene, methylbenzenes and other C_3-benzenes, naphthalene and methylnaphthalenes. Other than naphthalenes, a few other polynuclear aromatics were present in the WSF. Their concentrations were much higher than in the original condensate.

3.1.4 Exposure Water

Qualitatively, the hydrocarbon profiles of all the exposure water (seawater spiked with stock WSF) tested were similar to that of the stock WSF discussed above. For comparison, a GLC hydrocarbon profile of exposure water is shown in Figure 3.3. The GLC hydrocarbon profiles of all the exposure water analyzed during the five exposure trials are given in Appendix C. The chromatograms indicate that there is a considerable loss of some of the very volatile hydrocarbons after dilution. This is apparent with cyclohexane, methylcyclohexane and benzene.

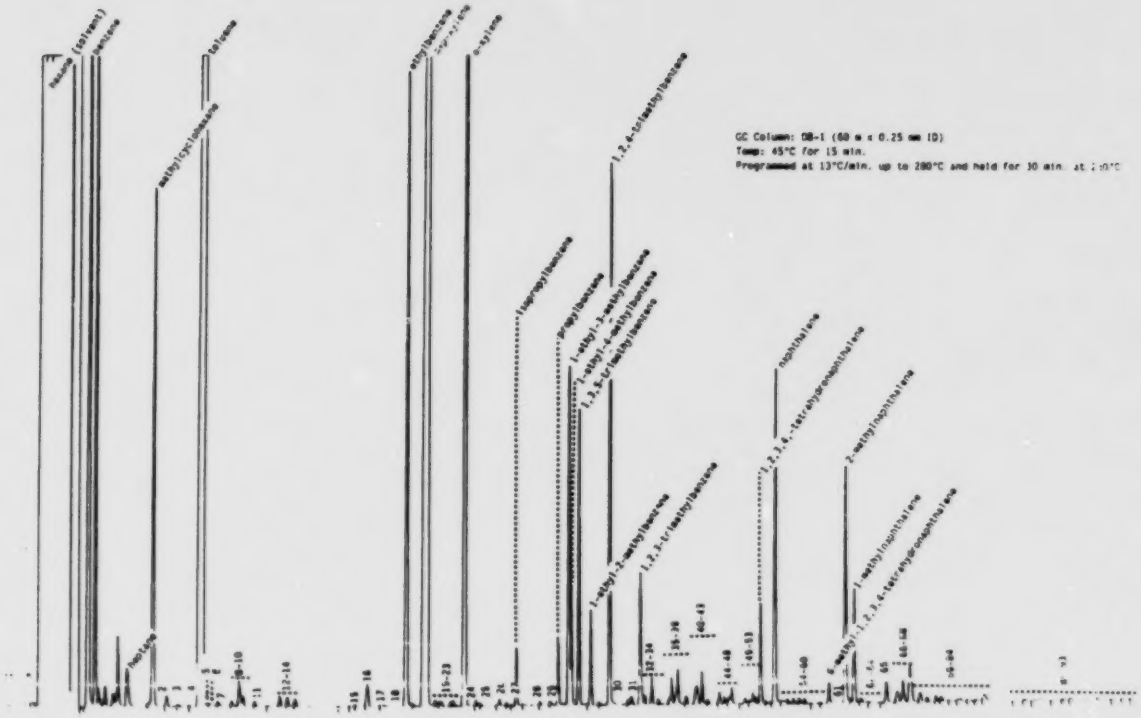


Figure 3.2. GLC hydrocarbon profile of stock WSF (56 ppm hydrocarbon - condensate). See Table 3.1 for identification of numbered peaks.

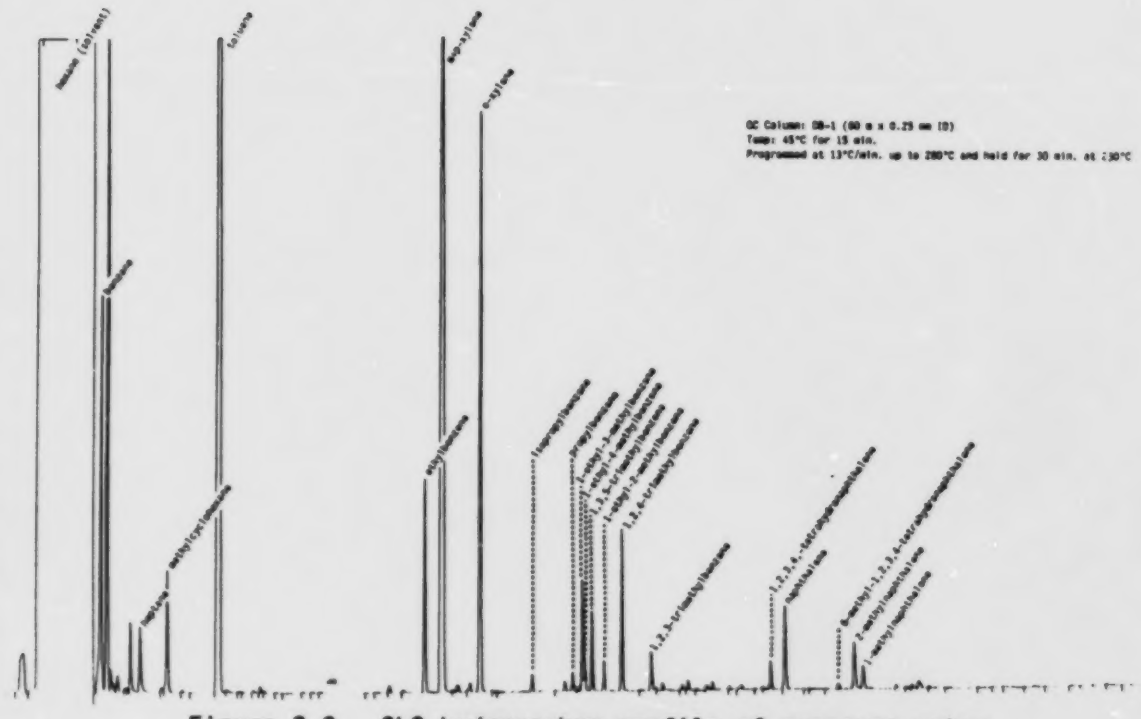


Figure 3.3. GLC hydrocarbon profile of exposure water (4.9 ppm hydrocarbon - condensate WSF).

Table 3.1. Hydrocarbons in the stock WSF prepared from condensate (see Figure 3.2).

Peak #	Retention Time (min)	Hydrocarbon	Major Fragments in MS (m/e)	Concentration in WSF (ppm)
1		pentane	---	
-	1.96	benzene	---	9.35
-	3.48	n-heptane	---	0.05
		iso-octane	---	1.95
-	4.42	methylcyclohexane	---	
2-4	4.96,5.41,5.83	unidentified	---	
-	6.38	toluene	---	30.00
5-8	6.68,6.78,7.08, 7.63	unidentified	---	
9-15	7.80,7.94,8.37, 9.31,9.70,9.81, 11.21	unidentified dimethylcyclohexanes	--- 55(100%),7,112(M^+)	
16	11.49	ethylcyclohexane	55(100%),83,110(M^+)	0.28
17,18	11.78,11.89	ethylcyclohexane	55(100%),83,110(M^+)	
-	12.42	ethylbenzene	---	1.19
-	12.91	m+p-xylene	---	8.41
19	13.13	2,2,3-trimethylhexane	56,57(100%),70,71(M^+)	
20,21	13.29,13.33	unidentified	---	
22	13.51	cyclooctane	56,67(100%),81,95,110(M^+)	
23	13.63	unidentified	---	
-	13.89	o-xylene	---	3.35
24	14.19	1-methyl-cis-4-ethylcyclohexane	58(100%),56,69,81,97,126(M^+)	
25,26	14.29,14.7	unidentified	---	
27	14.81	n-nonane	---	0.02
-	15.23	isopropylbenzene	51,65,77,79,91,105(100%),120(M^+)	0.09
28	15.83	n-propylcyclohexane	55(100%),67,82,83,96,126(M^+)	
29	16.15	unidentified	---	
-	16.32	propylbenzene	65,91(100%),105,120(M^+)	0.15
-	16.59	1-ethyl-3-methylbenzene	91,105(100%),120(M^+)	0.35
-	16.65	1-ethyl-4-methylbenzene	▪ ▪	0.10
-	16.85	1,3,5-trimethylbenzene	51,63,91,105(100%),120(M^+)	0.33
-	17.16	1-ethyl-2-methylbenzene	91,105(100%),120(M^+)	0.02
-	17.64	1,2,4-trimethylbenzene	51,63,91,105(100%),120(M^+)	0.60
30	18.09	iso-butylbenzene	65,91(100%),134(M^+)	] 0.02
31	18.17	sec-butylbenzene	91,105(100%)134(M^+)	
32	18.42	1,2,3-trimethylbenzene	77,91,105(100%),120(M^+)	0.08
33	18.55	4-isopropyl-1-methylbenzene	91,119(100%),134(M^+)	]
-	18.73	2-isopropyl-1-methylbenzene	91,119(100%),134(M^+)	
34	18.90	indane (2,3-dihydroindene)	51,58,63,74,91,115,117(100%),118((M^+)	
35	19.17	n-butylbenzene	77,91,105(100%),134(M^+)	
36	19.24	1-methyl-4-propylbenzene	91,105(100%),134(M^+)	

Cont.

Table 3.1 (Continued)

Peak #	Retention Time (min)	Hydrocarbon	Major Fragments in MS (m/e)	Concentration in WSF (ppm)
37	19.34	1,2-diethylbenzene		
38	19.40	1-methyl-2-propylbenzene	91,105(100%),134(M^+)	0.29
39	19.62	1,4-dimethyl-2-ethylbenzene	77,91,105(100%),134(M^+)	
40	19.86	2-methylindane	91,115,117(100%),132(M^+)	
-		dimethylethylbenzene	91,105,119(100%),134(M^+)	
41	19.90	dimethylethylbenzene	" "	
-		1-methylindane	91,115,117(100%),132(M^+)	
42	20.04	dimethylethylbenzene	91,105,119(100%),134(M^+)	
43	20.19	dimethylethylbenzene	" "	
44	20.50	dimethylethylbenzene	" "	
45	20.61	dimethylethylbenzene	" "	
46	20.70	C_4-benzene	91,105,115,119(100%),134(M^+)	
47	20.76	C_5-benzene	91,105,119(100%),133,148(M^+)	
48	20.83	C_5-benzene	" "	
49	21.04	3-methyl-3-phenyl-1-butene	91,105,115,131(100%),146(M^+)	0.22
50	21.19	dimethylstyrene	91,105,117(100%),132(M^+)	
51	21.30	C_5-benzene	91,105,119(100%),133,148(M^+)	
52	21.40	ethylstyrene	91,117(100%),132(M^+)	
53	21.47	C_5-benzene	91,105,119(100%),133,148(M^+)	
-	21.62	1,2,3,4-tetrahydronaphthalene	91,104(100%),115,117,128,132(M^+)	
-	22.00	naphthalene	102,128(100%,M^+)	0.67
54	22.31	C_6-benzene	91,105,119(100%),133,148(M^+)	
55	22.48	p-ethylcumene	91,105,119,113(100%),148(M^+)	
56	22.64	2-tetralone	91,104(100%),115,131,146(M^+)	
57	22.78	methylphenylbutene	91,117,131(100%),146(M^+)	
58	22.94	dimethylindane	77,91,115,116,129,131(100%),146(M^+)	
59	23.10	unidentified	---	0.08
60	23.21	unidentified	---	
-	23.45	6-methyl-1,2,3,4-tetrahydronaphthalene	77,81,105,115,117,131(100%),146(M^+)	
61	23.72	unidentified	---	
-	23.89	2-methylnaphthalene	115,139,141,142(100%,M^+)	0.32
-	24.15	1-methylnaphthalene	" "	0.13
62	24.33	unidentified	--	
63	24.52	unidentified	--	
64	24.80	unidentified	--	
65	25.04	diphenyl	51,63,76,102,105,126,127,128,152,153,154(100%,M^+)	
66	25.33	ethylnaphthalene	115,128,141(100%),156(M^+)	
67	25.50	dimethylnaphthalene	115,128,141,156(100%,M^+)	0.16
68	25.70	dimethylnaphthalene	" "	
69	25.70	dimethylnaphthalene	" "	
70	25.98	dimethylnaphthalene	" "	

cont.

Table 3.1 (Continued)

Peak #	Retention Time (min)	Hydrocarbon	Major Fragments in MS (m/e)	Concentration in WSF (ppm)
71	26.18	dimethylnaphthalene	" "	
72	26.18	dimethylnaphthalene	" "	
73	26.43	dimethylnaphthalene	65,82,91,152,153,165,167,168(100%,M^+)	
74	26.57	1,1-diphenylethane	115,141,152,153,165,167(100%)(MW182)	
75	26.57	trimethylnaphthalene	115,126,127,128,141,153,155(100%)(MW182)	
76	26.80	C_n-alkenylnaphthalene	63,84,114,139,153,155,168(100%,M^+)	
77	26.80	trimethylnaphthalene	115,126,127,128,141,153,155(100%),170(M^+)	0.07
78	27.08	trimethylnaphthalene	" "	
79	27.16	trimethylnaphthalene	115,126,127,128,141,153,155(100%),170(M^+)	
80	27.37	trimethylnaphthalene	" "	
81	27.61	trimethylnaphthalene	" "	
82	27.88	9h-fluorene	81,82,115,139,163,164,165,166(100%,M^+)	
83	28.03	3,4'-dimethyl-1,1'-biphenyl	153,155,165,167,183,182(100%,M^+)	
84-85	28.98	9h-fluorene,methyl	89,115,153,165(100%),180(M^+)	
		7-ethyl-1,4-dimethyl azulene	83,91,115,128,153,155,167(100%),184(M^+)	
		4-methyldibenzofuran	91,129,152,183,184(100%,M^+)	
		ethyl-1,1'-biphenyls	91,115,139,152,165,167(100%),181,182(M^+)	0.05
86-87	29.88	methyl-9h-fluorenes	152,165,180(100%,M^+)	
90	30.64	unidentified	--	
91	31.04	unidentified	--	
92	31.47	unidentified	--	
93	31.77	unidentified	--	
88	30.03	unidentified	--	
89	30.26	unidentified	--	

With the exception of the 96 h exposure, the total hydrocarbon concentrations, as well as those of the individual hydrocarbons, did not change significantly during the exposure periods. This was due to the fact that the exposure tanks were continuously fed with a slow stream of stock WSF and clean aerated seawater in order to maintain the hydrocarbon concentration at the desired level.

3.2 Toxicity Tests

The hydrocarbon exposure conditions and observed mortalities during the exposure and recovery phases of the toxicity tests are presented in Tables 3.2, 3.3 and 3.4.

There were no mortalities during the 48 h range-finding trial; however, there were mortalities during the following five-day recovery period. All the scallops exposed to 15.52 ppm died by 48 h in the recovery tanks, and 14% of the scallops from the 8.69 ppm exposures died by 120 h in the recovery tanks. Considering only mortalities during the WSF exposures, the 48 h LC50 is above the calculated 100% WSF concentration of 15.52 ppm. With the mortalities after the five day recovery included, the LC50 lies between 15.52 and 8.69 ppm hydrocarbon.

No mortalities were observed during the 24 h exposure; however, 20% from the 16.15 ppm exposure died by 120 h in the recovery tanks. The 24 h LC50 value is thus greater than the calculated 100% WSF concentration of 16.15 ppm.

Mortalities were observed during the 96 h WSF exposures. All of the scallops in the 21.26 ppm exposure died by 96 h of exposure. No scallops died during the other less concentrated exposures; however, 80% of the scallops from the 14.46 ppm exposures died by 120 h in the recovery tanks. Five percent of the scallops from the 9.78 ppm exposures died by 120 h in the recovery tanks.

Table 3.2. Adult scallop 48 hour rangefinding trials (condensate - water-soluble fraction).

Treatment			Mortalities								
(% WSF)		Hydrocarbon Concentration (ppm)[1]	n	Exposure Phase 24 h	Exposure Phase 48 h	Recovery Phase 24 h	Recovery Phase 48 h	Recovery Phase 72 h	Recovery Phase 96 h	Recovery Phase 120 h	Total
0 (control)	A	0	7	0	0	0	0	0	0	0	0
	B	0	7	0	0	0	0	0	0	0	0
10	A	1.55	7	0	0	0	0	0	0	0	0
	B	1.55	7	0	0	0	0	0	0	0	0
18	A	2.79	7	0	0	0	0	0	0	0	0
	B	2.79	7	0	0	0	0	0	0	0	0
32	A	4.97	7	0	0	0	0	0	0	0	0
	B	4.97	7	0	0	0	0	0	0	0	0
56	A	8.69	6	0	0	0	0	0	0	0	0
	B	8.69	7	0	0	0	0	0	0	2	2
100	A	15.52	7	0	0	0	7	-	-	-	7
	B	15.52	7	0	0	0	7	-	-	-	7

[1]Values calculated from measured initial hydrocarbon concentration (100% WSF) calibrated with fluorometer and GC (See Appendices D and F).

Table 3.3. Adult scallop 24 hour LC50 trials (condensate - water-soluble fraction).

Treatment			Mortalities							
(% WSF)		Hydrocarbon Concentration (ppm)[1]	n	Exposure Phase 24 h	Recovery Phase 24 h	48 h	72 h	96 h	120 h	Total
0 (control)	A	0	10	0	0	0	0	0	0	0
	B	0	10	0	0	0	0	0	0	0
22	A	3.55	10	0	0	0	0	0	0	0
	B	3.55	10	0	0	0	0	0	0	0
32	A	5.17	10	0	0	0	0	0	1	1
	B	5.17	10	0	0	0	0	0	0	0
46	A	7.43	10	0	0	0	0	0	0	0
	B	7.43	10	0	0	0	0	0	0	0
68	A	10.98	10	0	0	0	0	0	0	0
	B	10.98	10	0	0	0	0	0	0	0
100	A	16.15	10	0	0	0	0	0	2	2
	B	16.15	10	0	0	0	0	0	2	2

[1]Values calculated from measured initial hydrocarbon concentration (100% WSF) calibrated with fluorometer and GC (See Appendices D and F).

Table 3.4. Adult scallop 96 hour LC50 trials (condensate - water-soluble fraction).

Treatment			Mortalities										
(% WSF)		Hydrocarbon Concentration (ppm)[1]	n	Exposure Phase 24 h	48 h	72 h	96 h	Recovery Phase 24 h	48 h	72 h	96 h	120 h	Total
0 (control)	A	0	10	0	0	0	0	0	0	0	0	0	0
	B	0	10	0	0	0	0	0	0	0	0	0	0
22	A	4.68	10	0	0	0	0	0	0	0	0	0	0
	B	4.68	10	0	0	0	0	0	0	0	0	0	0
32	A	6.80	10	0	0	0	0	0	0	0	0	0	0
	B	6.80	10	0	0	0	0	0	0	0	0	0	0
46	A	9.78	10	0	0	0	0	0	0	0	0	0	0
	B	9.78	10	0	0	0	0	0	0	0	0	1	1
68	A	14.46	10	0	0	0	0	0	4	-	4	1	9
	B	14.46	10	0	0	0	0	0	2	-	3	2	7
100	A	21.26	10	0	0	0	10	-	-	-	-	-	-
	B	21.26	10	0	0	0	10	-	-	-	-	-	-

[1]Values calculated from measured initial hydrocarbon concentration (100% WSF) calibrated with fluorometer and GC (See Appendices D and F).

Using binomial analysis, the 96 h LC50 value was calculated to be 17.43 ppm (95% C.I.:14.46-21.26 ppm). If one includes delayed mortalities observed during the five-day recovery period in the LC50 calculation,the 96 h LC50 value drops to 12.54 ppm (95% C.I.:9.78-14.46 ppm).

Behavioural observations of the scallops during the five-day recovery period are shown in Tables 3.5 and 3.6. After the 24 h exposure to WSF, the scallops in the 16.15 ppm tank showed both delayed mortality and recovery, the latter indicated by animals with extended tentacles around the shell. In general, the average tentacle response of the scallops from the middle concentrations did not change very much during the five-day recovery period. During the recovery period after the 96 h bioassay, scallops from the 14.46 and 9.78 exposures had delayed mortalities. Scallops from the 4.68 ppm and 9.78 ppm exposures exhibited some recovery, while on average the tentacle response of the scallops from the 6.80 ppm exposures did not change during the five-day recovery period.

The hydrocarbon levels in the exposure tanks dropped markedly during the 24 h between replacements. The average decrease was 89.7% (n=11) after 24 h.

3.3 Tainting Trials

3.3.1 General

In the following section, each trial is identified by the exposure time and the hydrocarbon concentration in the exposure water. The concentration is expressed as a time-weighted average of the fluorometer data which have been calibrated with GC analysis of water samples (see Appendices D and E). Each data point is weighted (multiplied) by the length of time it represents. These time-concentrations are then summed

Table 3.5. Behaviour of scallops during five-day recovery phase following 24 h exposure to water-soluble fraction of condensate.

Treatment			Number With Extended Tentacles Around Shell					Number With Extended Tentacles Only at Hinge Region					Number With No Tentacles Showing				
(% WSF)		Hydrocarbon[1] Concentration (ppm)	24 h	48 h	72 h	96 h	120 h	24 h	48 h	72 h	96 h	120 h	24 h	48 h	72 h	96 h	120 h
0	A	0	10	10	10	10	10	0	0	0	0	0	0	0	0	0	0
	B	0	10	10	10	10	10	0	0	0	0	0	0	0	0	0	0
22	A	3.55	0	10	10	10	10	0	0	0	0	0	0	0	0	0	0
	B	3.55	0	10	10	10	10	0	0	0	0	0	0	0	0	0	0
32	A	5.17	0	10	9	9	8	0	0	1	0	1	0	0	0	1	0(1)[2]
	B	5.17	0	10	10	10	9	0	0	0	0	1	0	0	0	0	0
46	A	7.43	8	10	8	10	8	2	0	2	0	2	0	0	0	0	0
	B	7.43	9	9	9	9	8	1	1	1	1	2	0	0	0	0	0
68	A	10.98	5	6	6	7	3	5	4	4	3	7	0	0	0	0	0
	B	10.98	5	4	4	8	7	5	6	6	2	3	0	0	0	0	0
100	A	16.15	0	0	0	0	5	7	7	6	6	1	3	3	4	4	2(2)
	B	16.15	0	0	0	0	3	9	8	7	7	4	1	2	3	3	1(2)

[1]Values calculated from measured initial hydrocarbon concentration (100% WSF) calibrated with fluorometer and GC (See Appendices D and F).
[2]Bracketed values indicate mortalities.

Table 3.6. Behaviour of scallops during five-day recovery phase following 96 h exposure to water-soluble fraction of condensate.

Treatment			Number With Extended Tentacles Around Shell				Number With Extended Tentacles Only at Hinge Region				Number With No Tentacles Showing			
(% WSF)		Hydrocarbon[1] Concentration (ppm)	24 h	48 h	96 h	120 h	24 h	48 h	96 h	120 h	24 h	48 h	96 h	120 h
0	A	0	10	10	10	10	0	0	0	0	0	0	0	0
	B	0	10	10	10	10	0	0	0	0	0	0	0	0
22	A	4.68	9	9	10	10	1	1	0	0	0	0	0	0
	B	4.68	8	10	10	10	2	0	0	0	0	0	0	0
32	A	6.80	5	4	3	5	5	6	7	5	0	0	0	1
	B	6.80	5	8	6	6	5	2	4	4	0	0	0	0
46	A	9.78	0	0	0	0	4	10	10	9	6	0	0	1
	B	9.78	1	1	0	0	5	9	10	8	4	0	0	1(1)[2]
68	A	14.46	0	0	0	0	0	0	0	0	10	8(2)	2(8)	1(9)
	B	14.46	0	0	0	0	0	0	0	0	10	6(4)	5(5)	3(7)
100	A	21.26	-	-	-	-	-	-	-	-	-	-	-	-
	B	21.26	-	-	-	-	-	-	-	-	-	-	-	-

[1]Values calculated from measured initial hydrocarbon concentration (100% WSF) calibrated with fluorometer and GC (See Appendices D and F).
[2]Bracketed values indicate mortalities.

and divided by the total exposure time. In Figures 3.4 to 3.8, individual points represent the transformed fluorometric data and the lines represent the time-weighted average hydrocarbon concentration which identifies each trial. The temperature and dissolved oxygen conditions for each trial are presented in Appendix E.

3.3.2 Exposure Without Depuration

Exposures for taint evaluation consisted of 3, 24, and 96 h exposures to three distinct concentrations of hydrocarbons for each time period. Scallops from the three 24 h exposures were sub-sampled for tissue analysis for hydrocarbon content (results presented in Section 3.4.2). The hydrocarbon levels for exposures without depuration are shown in Figures 3.4 to 3.6.

The results of the taste panel evaluation are shown in Table 3.7. Scallops in all the 3 h trials were found to be tainted. The level of significance of the taint increased with increasing exposure concentration, as did the preference for the control sample. The two lowest exposure concentrations (0.45 and 0.78 ppm hydrocarbon) in the 24 h trials did not significantly taint the scallop tissue. The highest exposure concentration (3.79 ppm hydrocarbon), however, resulted in tainted scallop meats and the panelists indicated a definite preference for the control sample.

In the 96 h exposure trials, the two exposure concentrations which did not result in a positive identification of tainting in the 24 h exposure were repeated. In this case, the scallops exposed to the lowest hydrocarbon level (~0.5 ppm) were not tainted while those exposed to a slightly higher level (~0.8 ppm) were tainted. Scallops from the highest exposure concentration (1.41 ppm hydrocarbon) were found to be tainted and, in all cases, the panelists indicated that the control scallops were more acceptable than the exposed scallops.

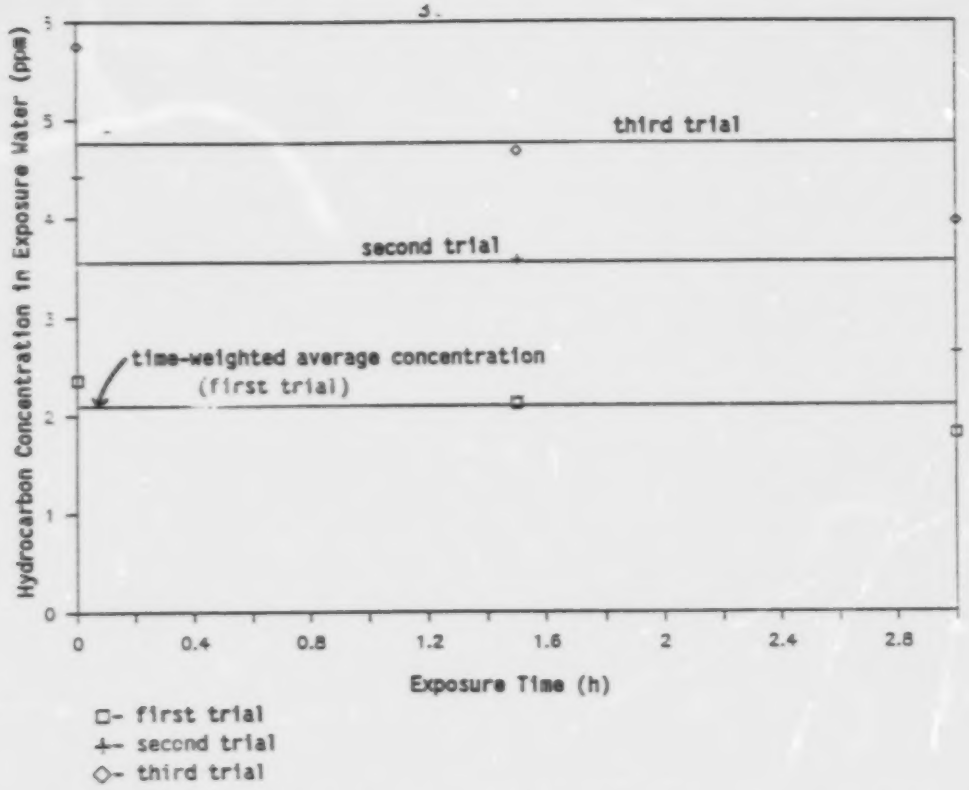


Figure 3.4. Hydrocarbon concentration in exposure water during 3 h trials.

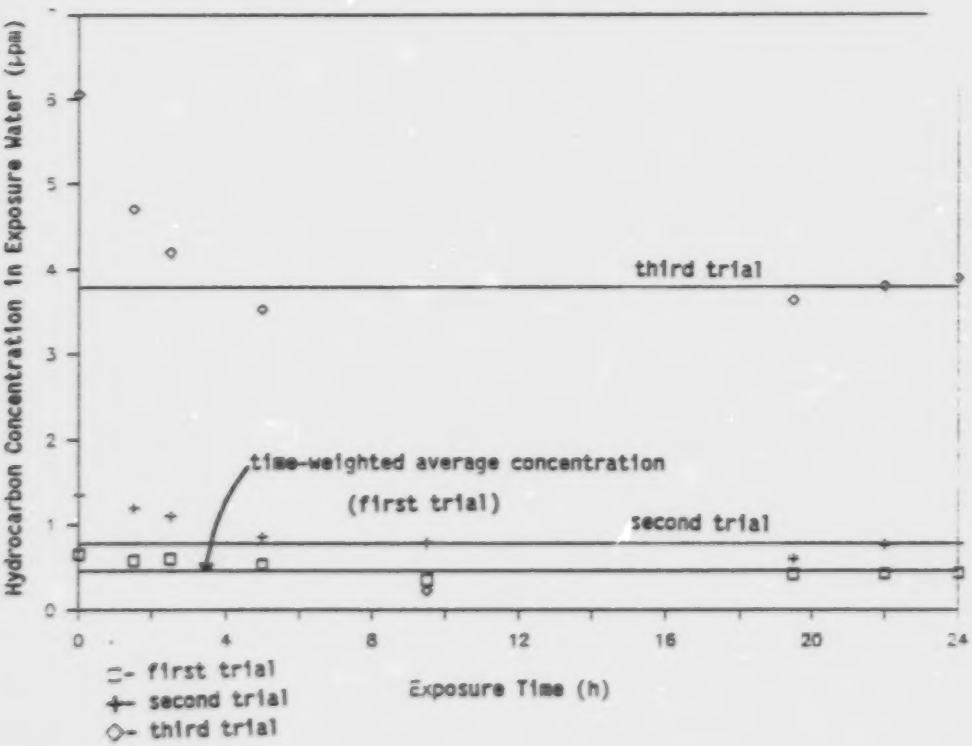


Figure 3.5. Hydrocarbon concentration in exposure water during 24 h trials.

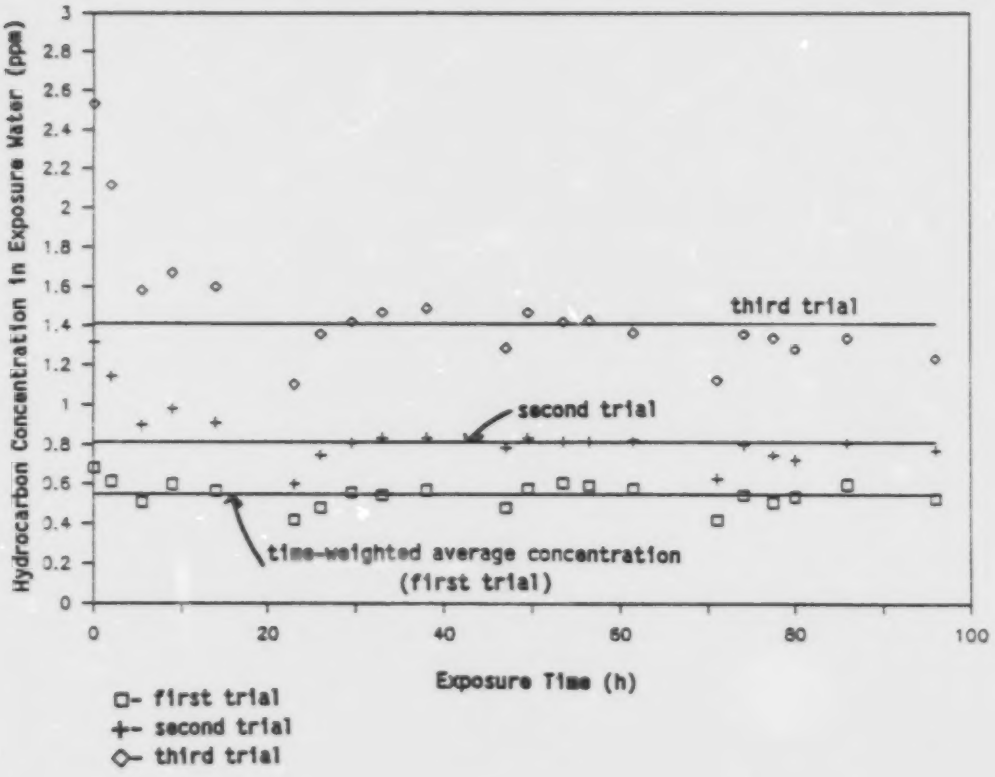


Figure 3.6. Hydrocarbon concentration in exposure water during 96 h trials.

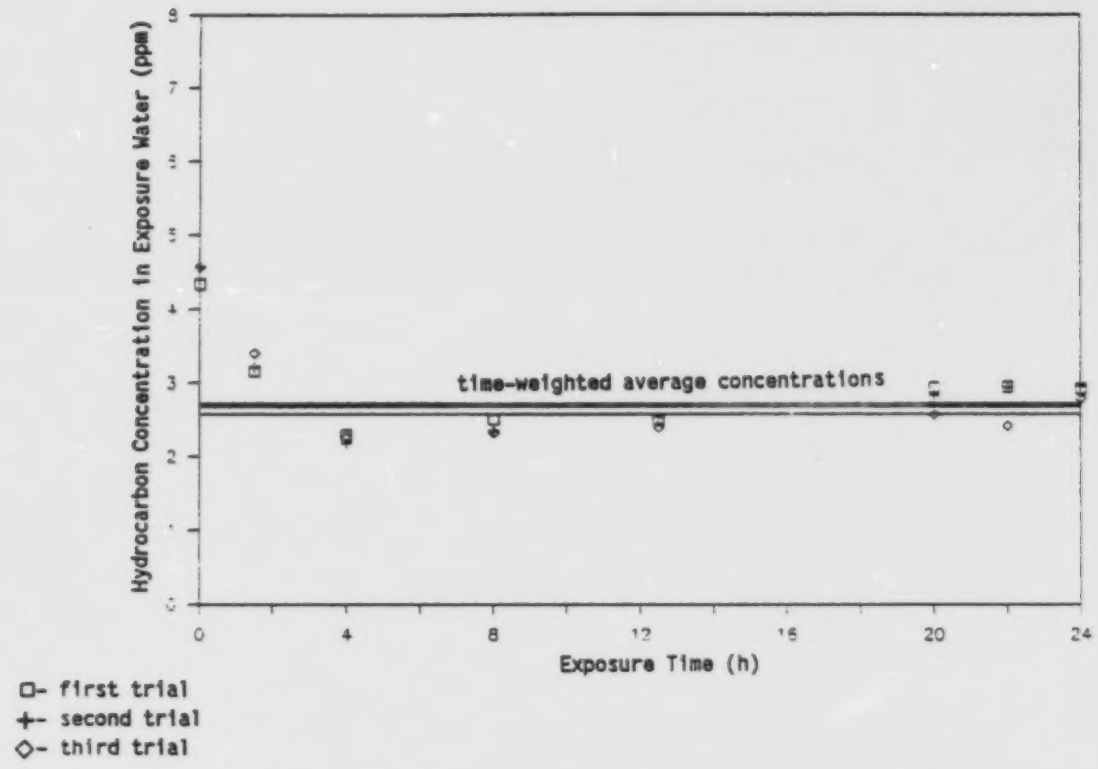


Figure 3.7. Hydrocarbon concentration in exposure water during depuration trials (24 h exposure period).

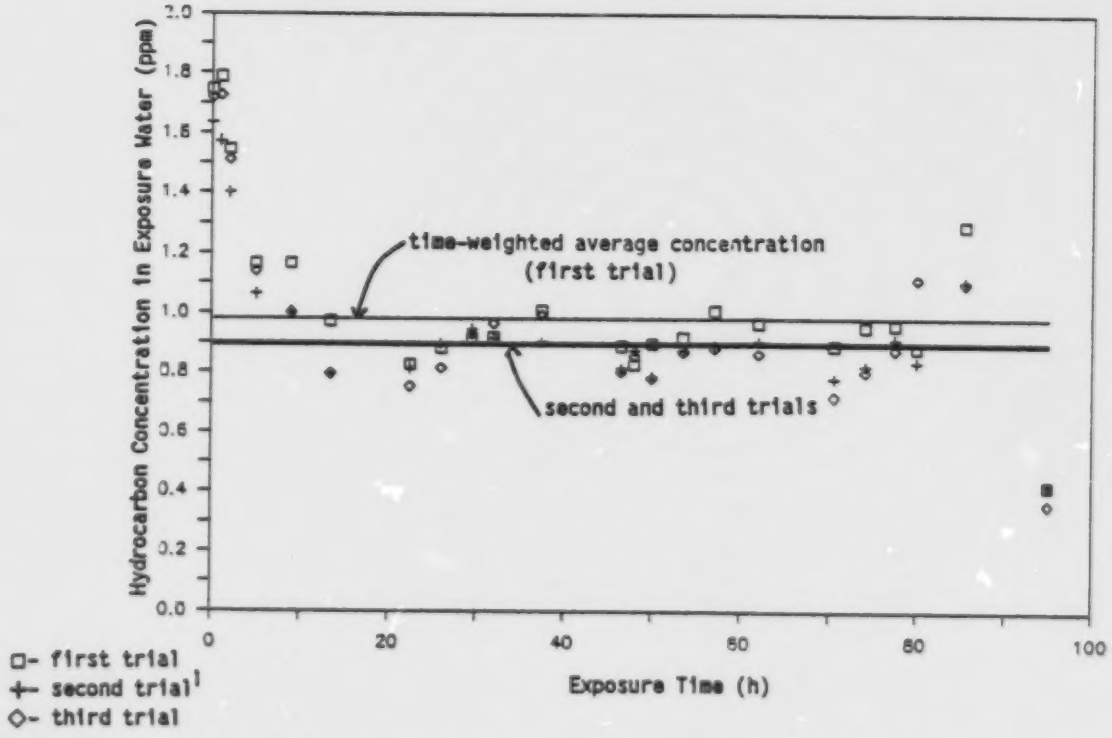


Figure 3.8. Hydrocarbon concentration in exposure water during depuration trials (96 h exposure period).

Table 3.7. Taste panel evaluation.

Trial		Detection of Taint			Preference for Control	
Exposure Time (h)	Hydrocarbon Exposure Level (ppm)	Number of Panelists	% Correctly[1] Identifying the Odd Sample	Level of Significance	% Preferring[3] the Control	Level of Significance
3	2.10	14	64	$p<.05$	78	$p<.01$
3	3.55	15	80	$p<.01$	100	$p<.001$
3	4.76	13	85	$p<.001$	100	$p<.001$
24	0.45	15	40	NS[2]	--	--
24	0.78	15	27	NS[2]	--	--
24	3.79	14	86	$p<.001$	100	$p<.001$
96	0.55	15	47	NS[2]	--	--
96	0.81	14	71	$p<.01$	80	$p<.01$
96	1.41	13	85	$p<.001$	100	$p<.001$

[1] In half of the cases, the odd sample was a control.
[2] Not significant.
[3] Percentage of those panelists who had correctly identified the odd sample.

The behaviour of the scallops in the exposure tanks was observed and compared to the behaviour of control scallops. The observed behaviour patterns generally fell into four main categories, as follows: position of valves, position of the tentacles, position of the mantle, and scallop movement. Activities within these main categories have been tabulated for each exposure trial at the beginning, mid-point, and end of each exposure period (Tables 3.8 to 3.10).

In all control trials, the scallops were observed to settle quietly to the bottom of the tank and partially open within the first few minutes. The mantles were well forward on the shell and the tentacles were extended. There was little or no movement in the tanks and the animals were never observed joined or clamped together. With increasing hydrocarbon exposure concentration and/or duration of exposure, the behaviour of the scallops shifted towards being more open, the tentacles and mantles retracted and, initially, increased activity. After prolonged exposure (>24 h) to relatively high hydrocarbon levels (>1 ppm) the level of activity was observed to decrease with the onset of individuals becoming clamped together.

3.3.3 Exposure Followed by Depuration

Two depuration trials were conducted for this project. The first consisted of a 24 h exposure to an average of 2.66 ppm hydrocarbon followed by a depuration phase lasting 216 h. For the second trial, scallops were exposed to an average of 0.92 ppm hydrocarbon for 96 h and allowed to depurate for up to 264 h. The hydrocarbon exposure conditions are summarized in Figures 3.7 and 3.8.

The taste panel results are shown in Table 3.11. Scallops were evaluated for the presence of a taint after 0, 24, 96 and 216 h of depuration in the first (24 h) exposure trial. The scallops were found to be significantly tainted after the exposure phase (i.e. 0 h depuration) with

Table 3.8. Behaviour of scallops during the 3 h exposure trials.

Trial		Behaviour											
Average Hydrocarbon Concentration (ppm)	Time of Exposure	Closed	Partially Open	Open Wide	Tentacles Extended	Tentacles Retracted	Mantle Extended	Mantle Retracted	Active Swimming	Slowly Clapping	Spinning	Little or no Movement	Clamped Together
2.10	beginning	*	*	*	*	*	*	*		*	*	*	
	middle		*		*	*	*	*		*	*		
	end		*	*	*	*	*						
3.55	beginning		*	*	*	*	*	*		*	*		*
	middle		*	*	*	*	*	*		*	*		*
	end		*	*	*	*	*	*					
4.76	beginning		*	*	*	*	*	*	*	*	*		*
	middle		*	*	*	*	*	*		*	*		*
	end												
0 (control)	beginning		*		*		*					*	
	middle		*		*		*					*	
	end		*		*		*					*	

* Behaviour observed in several or more scallops.

Table 3.9. Behaviour of scallops during the 24 h exposure trials.

Trial		Behaviour											
Average Hydrocarbon Concentration (ppm)	Time of Exposure	Closed	Partially Open	Open Wide	Tentacles Extended	Tentacles Retracted	Mantle Extended	Mantle Retracted	Active Swimming	Slowly Clapping	Spinning	Little or no Movement	Clamped Together
0.45	beginning	*	*		*		*					*	
	middle		*		*	*	*					*	
	end		*		*	*	*					*	
0.78	beginning	*	*		*		*		*				
	middle		*	*	*	*	*	*	*	*	*		
	end		*	*	*	*	*	*	*	*	*		
3.79	beginning	*	*		*	*	*	*		*	*		
	middle			*		*		*			*		
	end			*		*		*				*	*
0 (control)	beginning		*		*		*					*	
	middle		*		*		*					*	
	end		*		*		*					*	

* Behaviour observed in several or more scallops.

Table 3.10. Behaviour of scallops during the 96 h exposure trials.

Trial		Behaviour											
Average Hydrocarbon Concentration (ppm)	Time of Exposure	Closed	Partially Open	Open Wide	Tentacles Extended	Tentacles Retracted	Mantle Extended	Mantle Retracted	Active Swimming	Slowly Clapping	Spinning	Little or no Movement	Clamped Together
0.55	beginning		*		*	*	*	*				*	
	middle		*		*	*	*					*	
	end		*		*	*	*					*	
0.81	beginning		*	*	*	*	*					*	
	middle		*	*	*	*	*				*	*	
	end		*	*	*	*	*			*	*		
1.41	beginning		*	*	*	*	*	*	*	*			
	middle		*	*	*	*	*	*		*	*		*
	end		*	*		*	*	*		*	*		*
0 (control)	beginning		*		*		*					*	
	middle		*		*		*					*	
	end		*		*		*					*	

* Behaviour observed in several or more scallops.

Table 3.11. Taste panel evaluation - depuration trials.

Trial	Detection of Taint			Preference for Control	
24 h Exposure 2.66 ppm Hydrocarbon Depuration Time (h)	Number of Panelists	% Correctly[1] Identifying the Odd Sample	Level of Significance	% Preferring[3] the Control	Level of Significance
0	14	93	p<.001	100	p<.001
24	15	53	NS[2]	--	--
96	15	53	NS[2]	--	--
216	15	40	NS[2]	--	--

Trial	Detection of Taint			Preference for Control	
96 h Exposure 0.92 ppm Hydrocarbon Depuration Time (h)	Number of Panelists	% Correctly[1] Identifying the Odd Sample	Level of Significance	% Preferring[3] the Control	Level of Significance
0	15	60	p<.05	100	p<.001
24	13	85	p<.01	55	NS[2]
96	15	60	p<.05	78	p<.01
264	14	29	NS[2]	--	--

[1] In half of the cases, the odd sample was a control.
[2] Not significant.
[3] Percentage of those panelists who had correctly identified the odd sample.

all of the panelists who had correctly identified the odd sample preferring the control scallops. After 24 h depuration, however, the taste panel results indicated no significant tainting. This depuration of taint was confirmed by the results of the taste panels conducted with the scallops which had depurated for 96 and 216 h.

In the second trial, scallops were tested for taint after 0, 24, 96 h and 264 h of depuration. The scallops remained tainted until at least 96 h of depuration. While the level of significance for the detection of taint was low ($p<0.05$) for the scallops not depurated (0 h), all of the panelists who had correctly identified the odd sample preferred the control scallops. Conversely, after 24 h depuration, the level of significance for the detection of taint was greater ($p<0.01$); however, only 55% of those panelists actually preferred the control scallops. In this instance, the amount of control tissue available was less than the amount of exposed tissue and consequently the control samples became over cooked. It is likely that the panelists were able to identify the odd sample based on the degree of cooking alone, thus biasing the results. After 96 h depuration, the percentage of panelists correctly identifying the odd sample was the same (60%) as after 0 h depuration; however, fewer of the panelists preferred the control sample (78% compared to 100%).

The final sampling period was extended an additional 2 days (from 216 in the first depuration trial to 264 h) in order to be more assured of a depurated sample. The taste panel results of this sample (264 h) indicated no significant tainting with only 29% of the panelists correctly identifying the odd sample.

The behaviour patterns observed in scallops exposed for the depuration studies are presented in Table 3.12. The behaviour of the scallops in both cases suggests relatively high exposure levels. The scallops were active during the first half of the exposure phase

Table 3.12. Behaviour of scallops during depuration trials.

Trial		Behaviour											
Average Hydrocarbon Concentration (ppm)	Time of Exposure	Closed	Partially Open	Open Wide	Tentacles Extended	Tentacles Retracted	Mantle Extended	Mantle Retracted	Active Swimming	Slowly Clapping	Spinning	Little or no Movement	Clamped Together
2.66 (24 h exposure)	beginning		*		*		*	*	*	*	*		
	middle		*	*	*	*	*	*		*	*		*
	end		*	*		*	*	*		*	*		*
0 *(24 h control)*	beginning		*		*		*					*	
	middle		*		*		*					*	
	end		*		*		*					*	*
0.92 (96 h exposure)	beginning		*	*	*	*	*		*	*	*		
	middle		*	*		*	*			*	*	*	
	end		*	*		*	*			*	*	*	*
0 (96 h control)	beginning		*		*		*					*	
	middle		*		*		*					*	
	end		*		*		*					*	

* Behaviour observed in several or more scallops.

resulting in individuals becoming clamped together. Several scallops (less than 4% of the total) were dead after the exposure phase. The scallops were gently pried apart and put in depuration tanks. Over the next 24 h, the behaviour of the scallops returned to that observed in the control groups, with the animals partially opening, mantles coming forward on the shell, and tentacles being extended. There was little or no movement.

3.4 Hydrocarbon Analysis of Scallop Adductor Muscle

3.4.1 Hydrocarbons in Control Scallops

Several subsamples of scallop adductor muscle from control groups were analyzed for hydrocarbon content. The chromatogram showed a number of very minor peaks as illustrated in Figure 3.9. Few of the peaks had the same retention times as those of the petrogenic hydrocarbons in the exposed samples (Section 3.4.2) and GC/MS analysis indicated that none of these peaks were petrogenic hydrocarbons. Most of these background peaks had molecular weights greater than 350. Comparison of the mass-spectral fragmentation pattern with the built-in mass-spectral library indicated that these peaks probably were alkyl silanes. The liquid phase of the capillary column used in the GC was a bonded methyl-silicone (DB-1) and thus it is possible that these background alkyl silanes are due to slight bleeding of this methyl-silicone column. It is known that bonded silicas with octadecyl groups used in chromatography columns slowly bleed alkyl silanes (Anthoni et al., 1987).

3.4.2 Hydrocarbons in Exposed Scallops

Subsamples of scallop adductor muscle from the 24 h trials, from all sampling times in the 24 h exposure and depuration trial, and from the last, depurated group after the 96 h exposure trial were analyzed for hydrocarbons. The results are shown in Table 3.13 to 3.15. As indicated in Appendix H, hydrocarbon recoveries from scallop adductor muscle were

Figure 3.9. GLC hydrocarbon profile of scallop adductor muscle not exposed to hydrocarbon.

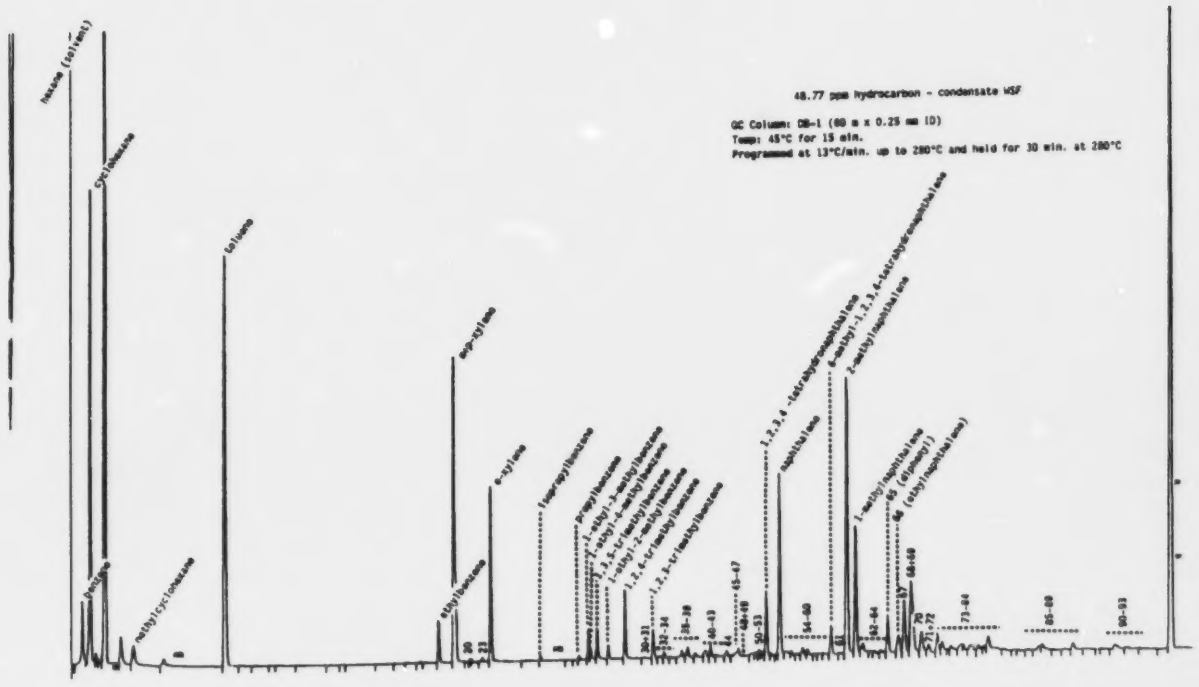


Figure 3.10. GLC hydrocarbon profile of scallop adductor muscle exposed for 24 h to 3.79 ppm hydrocarbon-condensate WSF.

Table 3.13. Total hydrocarbon levels in scallop adductor muscle exposed for 24 h to condensate WSF.

Hydrocarbon Exposure Level (ppm)	Hydrocarbon Level in Adductor Muscle (ppm)	Level of Significance of Taint
0.45	0.70	not significant
0.78	1.38	not significant
3.79	48.77	$p<0.001$

Table 3.14. Total hydrocarbon levels in scallop adductor muscle exposed for 24 h to 2.66 ppm hydrocarbon and depurated in clean seawater.

Depuration Time (h)	Hydrocarbon Level in Adductor Muscle (ppm)	Level of Significance of Taint
0	15.59	$p<0.001$
24	1.25	not significant
96	0.68	not significant
216	0.60	not significant

Table 3.15. Total hydrocarbon level in scallop adductor muscle exposed for 96 h to 0.92 ppm hydrocarbon and depurated in clean seawater for 11 days.

Depuration Time (h)	Hydrocarbon Level in Adductor Muscle (ppm)	Level of Significance of Taint
264	0.12	not significant

low, although reproducible. Because of this, the data on hydrocarbon levels in the adductor muscle can only be considered semi-quantitative.

The hydrocarbon concentrations in exposed scallop adductor muscles ranged from 0.70 to 48.77 ppm, dependent on the hydrocarbon concentration in the exposure water (Figures 3.10 to 3.14). With increasing depuration, there was a corresponding decrease in the hydrocarbon concentration in scallop adductor muscle (Figures 3.13 and 3.14). Only those adductor muscles with a hydrocarbon level exceeding 15 ppm were tainted.

Several aromatic compounds and a few saturated aliphatic hydrocarbons were detected in adductor muscle exposed to condensate WSF. The components identified by mass spectra and with gas chromatography retention times are shown in Figure 3.10 and Table 3.16. These hydrocarbons were not detected in the control group of scallops (see Figure 3.9).

The gas chromatogram patterns of the hydrocarbons in adductor muscles exposed to low concentrations of WSF were qualitatively very similar to each other (Figures 3.11 and 3.12) and to the exposure water samples. As in the exposure water samples, the major hydrocarbons in the adductor muscle of tainted scallops were toluene and xylenes. However, there were quantitative differences between the adductor muscle and the exposure water hydrocarbons, especially in the higher alkylbenzenes (C_3,C_4 benzenes) and the alkylnaphthalene region. Higher molecular weight aromatics, especially naphthalene, methylnaphthalene, and dimethylnaphthalene, which are present in relatively low concentrations in the exposure water, compared to toluene and xylene, were found to be important components in the adductor muscle. This is quite evident in the scallops exposed to 3.79 ppm hydrocarbon (Table 3.16). Further, a few aromatic compounds including polynuclear aromatics, 1,1'-biphenyl, 5-methyl-1,2,3,4-tetrahydronaphthalene, C_2-naphthalenes, and fluorenes, which were present in trace levels in the WSF, were detected in the exposed adductor muscles as very minor components.

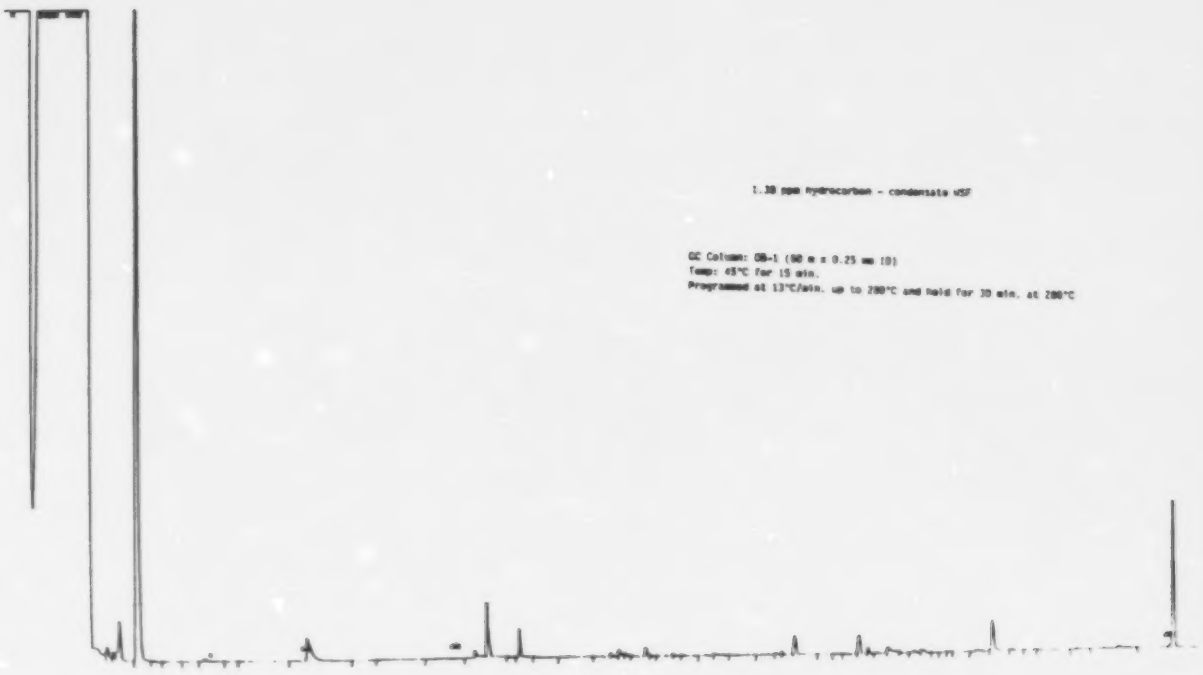


Figure 3.11. GLC hydrocarbon profile of scallop adductor muscle exposed for 24 h to 0.78 ppm hydrocarbon-condensate WSF.

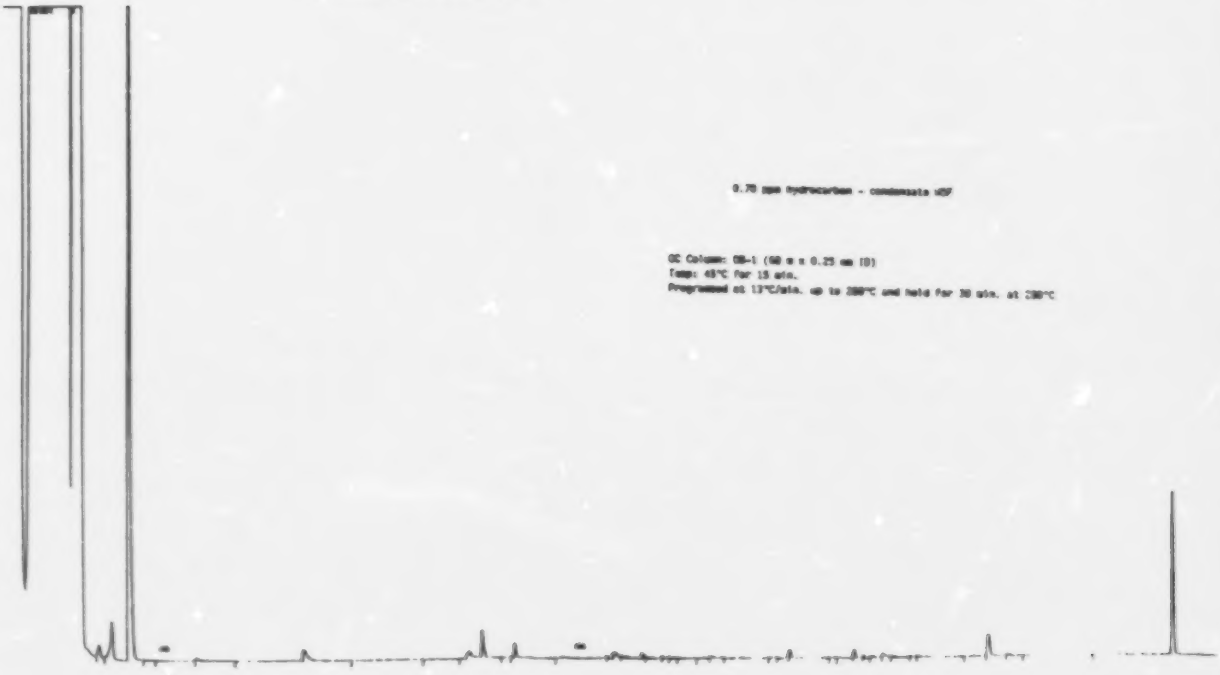


Figure 3.12. GLC hydrocarbon profile of scallop adductor muscle exposed for 24 h to 0.45 ppm hydrocarbon-condensate WSF.

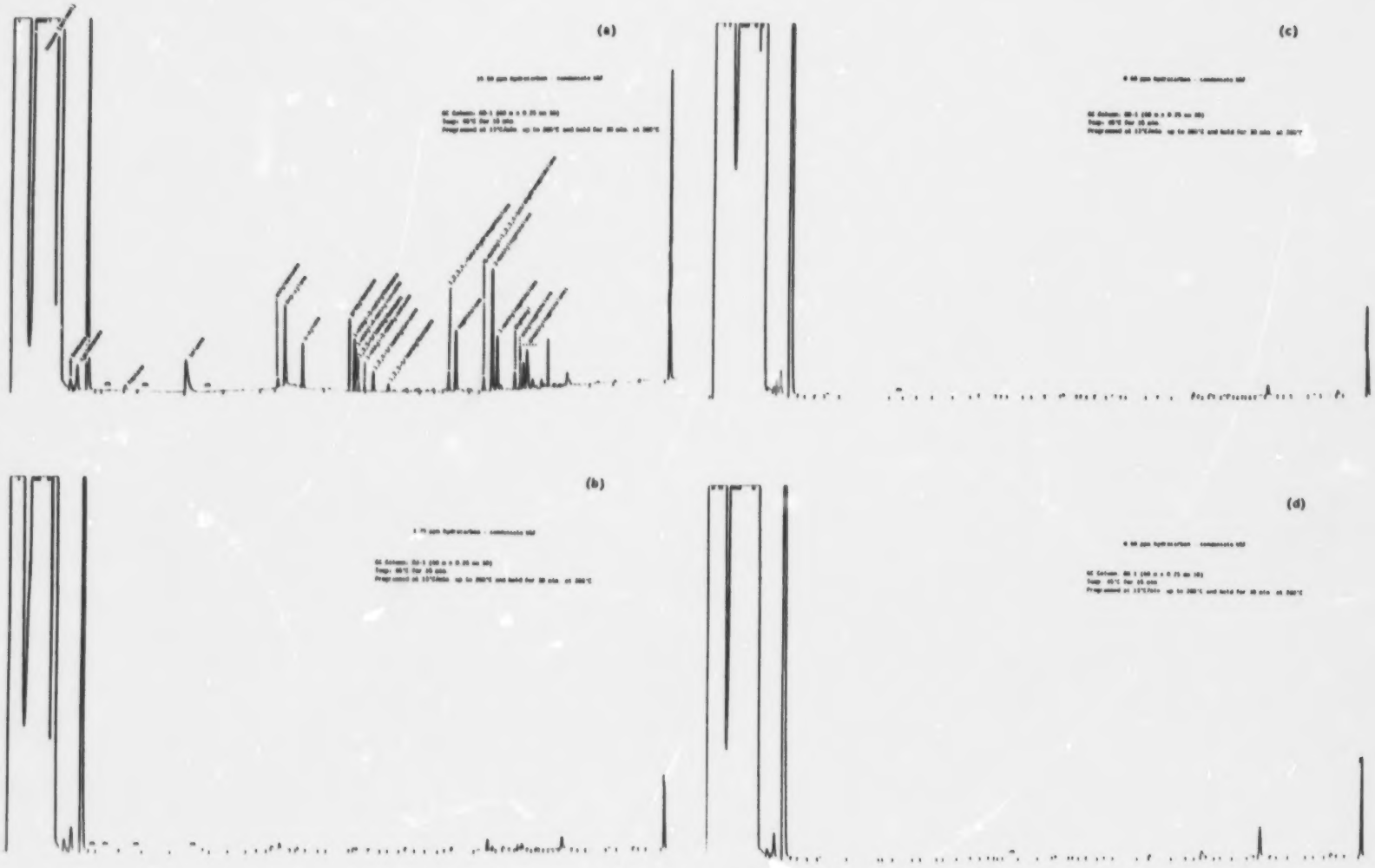


Figure 3.13. GLC hydrocarbon profiles of scallop adductor muscle exposed for 24 h to 2.66 ppm hydrocarbon-condensate WSF and depurated for: (a) 0 h, (b) 24 h, (c) 96 h, and (d) 216 h.

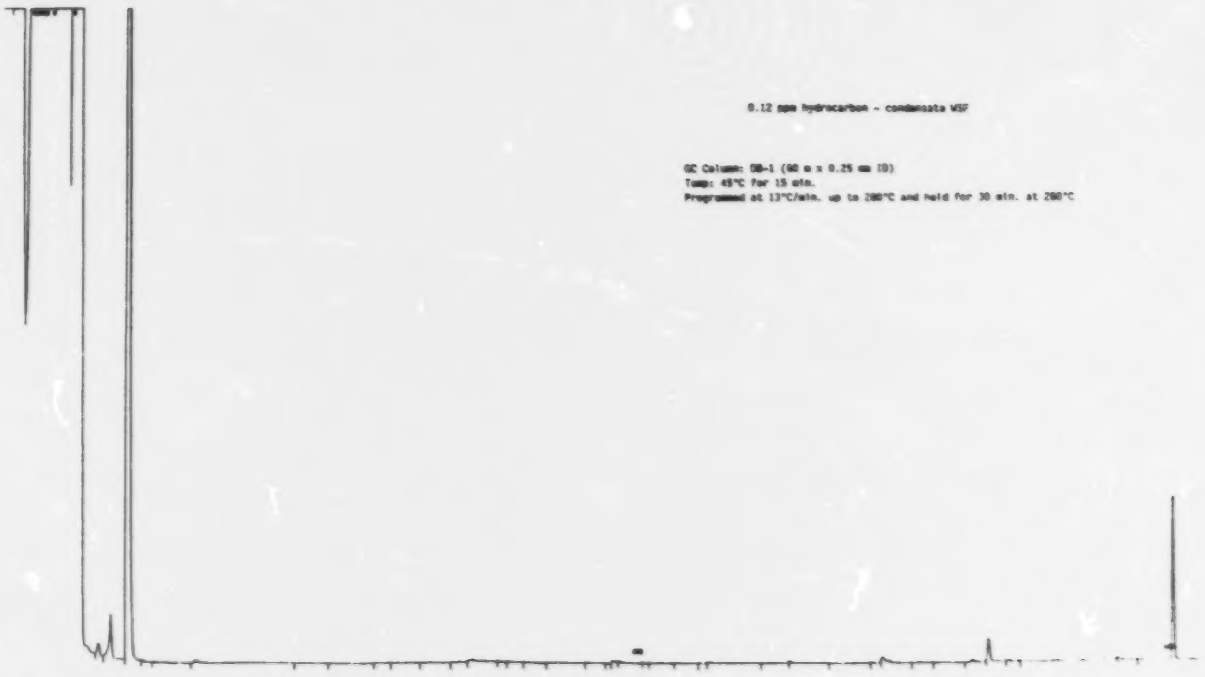

0.12 ppm hydrocarbon - condensate WSF
GC Column: DB-1 (90 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and held for 30 min. at 280°C

Figure 3.14. GLC hydrocarbon profile of scallop adductor muscle exposed for 96 h to 0.92 ppm hydrocarbon-condensate WSF and depurated for 264 h.

Table 3.16. Hydrocarbon composition of scallop adductor muscle exposed for 24 h to 3.79 ppm hydrocarbon.

Peak #[1]	Hydrocarbon	Concentration in Adductor Muscle (ppm)
-	benzene	1.75
-	n-heptane	1.03
	iso-octane	0.08
-	methylcyclohexane	0.28
-	toluene	14.41
5-8	unidentified	
9-15	unidentified	0.06
	dimethylcyclohexanes	
16	ethylcyclohexane	
17,18	ethylcyclohexanes	
-	ethylbenzene	1.25
-	m+p-xylene	5.29
19	2,2,3-trimethylhexane	
20,21	unidentified	
22	cyclooctane	
23	unidentified	0.16
-	n-nonane	
24	1-methyl-cis-4-ethylcyclohexane	
25,26	unidentified	
27	o-xylene	3.14
-	isopropylbenzene	0.10
28	n-propylcyclohexane	
29	unidentified	0.02
-	propylbenzene	0.32
-	1-ethyl-3-methylbenzene	0.57
-	1-ethyl-4-methylbenzene	0.11
-	1,3,5-trimethylbenzene	0.78
-	1-ethyl-2-methylbenzene	0.38
-	1,2,4-trimethylbenzene	1.60
32	1,2,3-trimethylbenzene	0.67
33	4-isopropyl-1-methylbenzene	
-	2-isopropyl-1-methylbenzene	
34	indane (2,3-dihydroindene)	
35	n-butylbenzene	
36	1-methyl-4-propylbenzene	
37	1,2-diethylbenzene	
38	1-methyl-2-propylbenzene	

Cont.

Table 3.16. (Continued)

Peak #[1]	Hydrocarbon	Concentration in Adductor Muscle (ppm)
39	1,4-dimethyl-2-ethylbenzene	1.20
40	2-methylindane	
-	dimethylethylbenzene	
41	dimethylethylbenzene	
-	1-methylindane	
42	dimethylethylbenzene	
43	dimethylethylbenzene	
44	dimethylethylbenzene	
45	dimethylethylbenzene	
46	C_4-benzene	
47	C_5-benzene	
48	C_5-benzene	
49	3-methyl-3-phenyl-1-butene	2.06
50	dimethylstyrene	
51	C_5-benzene	
52	ethylstyrene	
53	C_5-benzene	
-	1,2,3,4-tetrahydronaphthalene	
-	naphthalene	2.37
54	C_5-benzene	
55	p-ethylcumene	
56	2-tetralone	
57	methylphenylbutene	0.78
58	dimethylindane	
59	unidentified	
60	unidentified	
-	6-methyl-1,2,3,4-tetrahydronaphthalene	
61	unidentified	
-	2-methylnaphthalene	3.70
-	1-methylnaphthalene	1.64
62	unidentified	
63	unidentified	
64	unidentified	
65	diphenyl	
66	ethylnaphthalene	
67	dimethylnaphthalene	3.78
68	dimethylnaphthalene	
69	dimethylnaphthalene	
70	dimethylnaphthalene	
71	dimethylnaphthalene	
72	dimethylnaphthalene	
73	dimethylnaphthalene	
74	1,1-diphenylethane	
75	trimethylnaphthalene	
76	C_3-alkenylnaphthalene	

Cont.

Table 3.16. (Continued)

Peak #[1]	Hydrocarbon	Concentration in Adductor Muscle (ppm)
77	trimethylnaphthalene	0.74
78	trimethylnaphthalene	
79	trimethylnaphthalene	
80	trimethylnaphthalene	
81	trimethylnaphthalene	
82	9h-fluorene	
83	3,4'-dimethyl-1,1'-biphenyl	
84-85	9h-fluorene,methyl 7-ethyl-1,4-dimethyl azulene 4-methyldibenzofuran ethyl-1,1'-biphenyls	
86-87	methyl-9h-fluorenes	0.50
90	unidentified	
91	unidentified	
92	unidentified	
93	unidentified	
88	unidentified	
89	unidentified	
TOTAL		48.77

[1]See Figure 3.10 for location of peaks in the chromatogram.

4.0 DISCUSSION

4.1 Nature of the Scotian Shelf Condensate

The GC and GC/MS analytical work on the condensate provided for this study indicated that the condensate had undergone only minimal weathering prior to the study. The volatile aromatic components which are normally lost through evaporation during the initial stages of weathering comprised a significant fraction of the condensate. This was essential for proper evaluation of its tainting and toxic potential. The analysis also confirmed the generally conservative chemical composition of condensate, with toluene, xylenes, and alkylbenzenes dominating the aromatic fraction, similar to Maui and Uniacke condensates (Power, 1980; Carter, 1984).

The high aromatic content of the condensate contributed to its relatively high solubility compared to various crude oils (Ernst et al., 1988a,b). WSF concentrations as high as 56-95 ppm (depending on the mixing ratio) were achieved during this study, compared to 67 ppm observed in earlier studies (Hutcheson et al., 1983). Previous studies of the WSF of various crude oils made with the same apparatus used in the current study produced WSF concentrations of about 15 to 20 ppm (Ernst et al., 1988a,b).

The relatively high volatility of the condensate WSF was observed during the toxicity trials. Mild aeration in the exposure buckets resulted in a loss of almost 90% of the aromatic fraction measured by fluorescence in a 24-h period. The consequence of this is that the mean exposure concentration in the toxicity trials was probably only 30% to 50% of the initial measured concentration. Losses due to volatility were less of a problem in the tainting exposures because of the continuous stream of fresh condensate WSF into the exposure tanks.

The relatively high volatility of the condensate WSF was also reflected in the recovery data for the microextraction technique. For the lighter aromatic compounds, the recoveries were quite low. However, the repeatibility of the recoveries with the microextraction technique was very good, indicating a high level of confidence in the analytical data, once the correction factors had been applied.

The WSF of the Scotian Shelf condensate was qualitatively very similar to WSFs of various crude oils prepared in the same manner (Ernst et al., 1988a,b). This suggests that, despite differences in the original composition of various crude oils and condensates, it may be possible to assume a generic approach to the environmental implications of the WSFs of these various hydrocarbons. The tainting data discussed below appear to bear this out. Future studies involving exposure of animals to WSFs of oil and condensate should perhaps focus on different animals, depending on the region of concern, rather than on different types of oil or condensate.

4.2 Toxicity of the Condensate Water-Soluble Fraction

The toxicity data indicated total mortality of scallops exposed to 21.3 ppm condensate WSF for 96 h. The 96 h LC50 was 17.4 ppm. With delayed mortalities included, it was 12.5 ppm. All these data express the initial concentration of the WSF, not the mean exposure level. Considering the high evaporative loss of the measured fluorescing aromatic compounds that was observed over a 24 h period, the mean exposure level equivalent to a 96 h LC50 (including delayed mortality) could have been as low as 6-7 ppm. However, as most of the literature cites the initial concentration as the reference exposure level, comparisons must be made with the 96 h LC50 value of 12.5 ppm.

Although the LC50 value is put in the context of other toxicity data on condensate, it is important to point out that the use of different

extraction methods, standards, and analytical techniques makes the absolute comparison of toxicity data somewhat tenuous. This was clearly shown in the current project, in which the use of a different solvent and different excitation and emission wavelengths for fluorescence measurements resulted in a five fold difference in the analytical result, even though the same standard was used (see Appendix F).

Previous toxicity studies with estuarine and marine species involving the WSF of condensate indicate a range of 96 h LC50 values (initial concentration) as follows:

0.3 - 3 ppm - stickleback (E.P.S., 1983)
3.8 - 5.2 ppm - mummichog (Mahon et al., 1987)
0.9 - 5.6 ppm - trout (E.P.S., 1983; Hutcheson et al., 1983)
>23 ppm - sea urchin (Hutcheson et al., 1983)

The 96 h LC50 of 12.5 ppm for sea scallops exposed to condensate WSF falls into a logical position between fish and sea urchins. Sea scallops are apparently less sensitive to the toxic components of condensate WSF than the various fish species which have been tested.

4.3 Development and Persistence of Taint in Sea Scallops

4.3.1 The Exposure System as a Factor

The main goal of this part of the study was to examine the variables of exposure time and hydrocarbon concentration in the exposure water as factors which can influence the degree of tainting in scallops. Depuration time was also examined. In order to isolate time and hydrocarbon concentration as the determinant variables, features of the exposure system should not interfere with the interpretation of the exposure and taste panel results.

The exposure water temperature was maintained within ± 2°C during individual trials and the dissolved oxygen levels were maintained above 60% saturation as recommended by GESAMP (1983) and ECETOC (1987) (see Appendix E). The holding conditions during exposure were therefore not a source of stress which could introduce variability into the taste panel and analytical results.

The temperature of the exposure water varied only slightly (no more than 3°C) between trials as the ambient water temperature increased in late winter and early spring. The scallops, however, were acclimated to this water temperature regime and were never subjected to sudden temperature differences which could affect their behaviour in the exposure system. In addition, for each experimental group that was tested for tainting, there was a control reference group exposed to the same environmental conditions. Thus it would appear that comparisons of data within trials and between trials are legitimate.

In some of the exposure trials, the exposure water occasionally became super-saturated with oxygen (see Appendix E). It was felt that exposing scallops to super-saturated water intermittently for relatively short periods would not adversely stress them. Gas bubble trauma in bivalves is characterized by formation of gas blisters in soft body tissues and buoyancy of the whole animal (Bisker and Castagna, 1987). None of these symptoms were observed and there was no apparent mortality due to the exposure conditions. The behaviour of scallops occasionally exposed to super-saturated water was similar to the behaviour of scallops held in the normal unsaturated seawater supply.

The exposure system was designed to expose the large number of scallops needed to conduct a taste panel, while conserving the volume of WSF required to maintain the proposed exposure levels. With the exception of the long-term exposure trial, exposure time and hydrocarbon levels were stable within any one trial (see Appendix E), but varied between trials. As such, they are the only variables discussed in relation to tainting of scallops.

4.3.2 Tainting Threshold Level of Hydrocarbons in Exposure Water

Figure 4.1 summarizes for the purpose of discussion the hydrocarbon exposure and taste panel results. In this figure, the time-weighted average hydrocarbon exposure level in the seawater is examined in relation to the percentage of panelists who had correctly identified the odd sample from that exposure group, to gain some insight into the relationship between the two. The exposure time is identified by symbol and the levels of significance of the taste panel results have been separated into categories ranging from "not significant" to highly significant ($p<0.001$).

Scallops from trials in which the exposure level was $\leq$0.78 ppm hydrocarbon were not tainted, while scallops from exposure trials $\geq$0.81 ppm hydrocarbon were considered tainted, regardless of the duration of the exposure. Increasing the exposure time for any particular hydrocarbon exposure level tended to increase the percentage of correct responses from the panelists; however, at least with the lower exposure levels, the exposure time did not appear to influence whether or not a test group was tainted. For example, scallops exposed to 0.45 ppm hydrocarbon for 24 h were not tainted. Exposing scallops to approximately the same level (0.55 ppm) for an additional 64 h still did not result in a positive test for taint (Figure 4.1). Around the threshold level of 0.8 ppm hydrocarbon, it is possible that exposure time is a more significant factor in the acquisition of a taint as increasing the exposure time (from 3 h to 96 h) at similar hydrocarbon exposure levels results in a significant degree of tainting. Above an exposure level of 1.5 ppm, the degree of taint is so strong that the variables of exposure level and duration appear to have little effect on the perception of tainting.

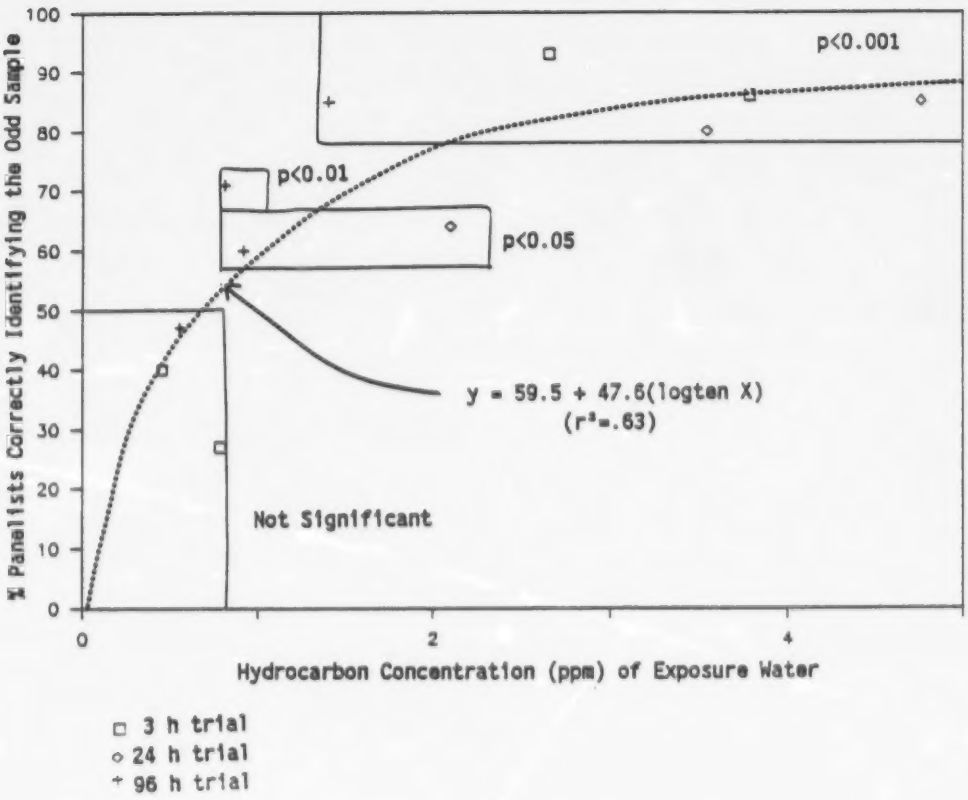


Figure 4.1. Relationship between taste panel results and hydrocarbon concentration of the exposure water.

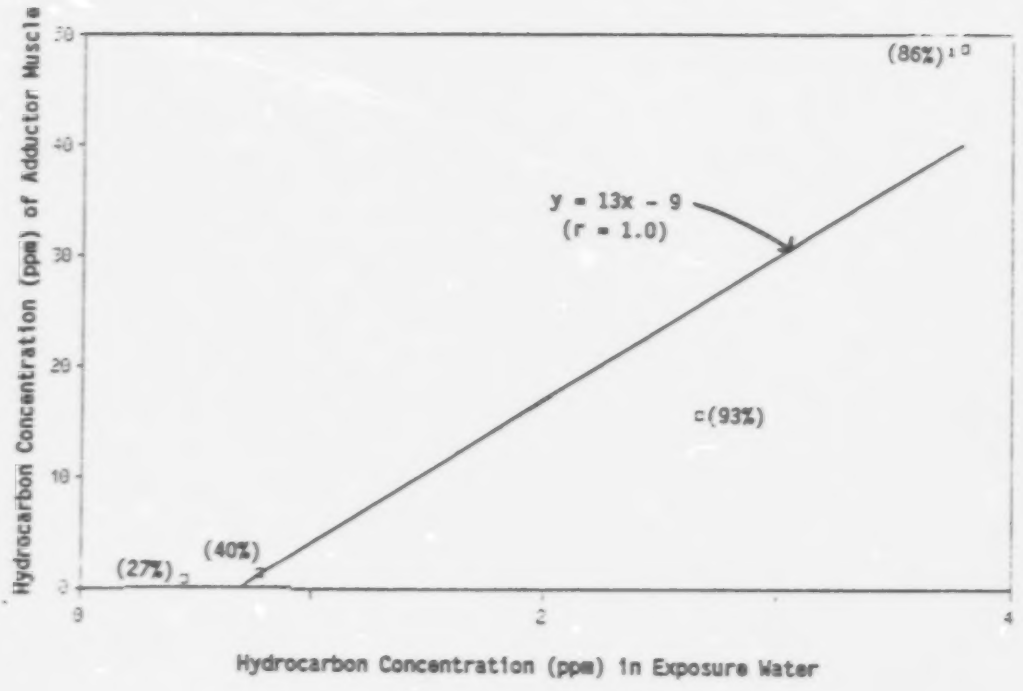
These results indicate that the threshold level of exposure to the WSF of Scotian Shelf condensate which results in a taint is about 0.8 ppm for exposure periods between 3 and 96 h. This value is very close to the threshold level of 0.5 ppm for scallops exposed to the WSF of Ninian crude oil (Ernst et al., 1988b). This comparison reinforces the observation of the similarity between WSFs of crude oil and condensate.

The observation that hydrocarbon concentration may be a more important factor than exposure time (at least over a period of 4 days) in inducing taint suggests that the tainting threshold reflects some aspect of scallop metabolism involved in uptake and excretion of hydrocarbons. It is possible that at hydrocarbon concentrations below 0.8 ppm, scallops can maintain an equilibrium between continuous dosing and excretion of the hydrocarbons such that no accumulation occurs in the adductor muscle. At higher exposure levels, the metabolic process responsible for hydrocarbon excretion may not work in equilibrium, and accumulation of hydrocarbons in the adductor muscle must occur, resulting in tainting.

The correlation between hydrocarbon exposure level and the degree of taint (r^2=0.6) can be expressed as an exponential curve. This lends some predictive quality to the results of this study which may be useful in assessing the potential for tainting in other low-lipid animals exposed to the WSF of condensate.

4.3.3 Tainting Threshold Levels of Hydrocarbons in the Adductor Muscle and Components Responsible for Tainting

Figure 4.2 shows the hydrocarbon levels in the scallop adductor muscle in relation to the hydrocarbon level in the exposure water and the respective taste panel results. For scallops exposed to low concentrations of condensate WSF (0.45 to 0.78 ppm) for 24 h, the level of accumulation of hydrocarbons in the adductor muscle was only 1.6 to 1.8 x the level in the water and these muscles were not tainted (Table 4.1). At



[1]% panelists correctly identifying the odd sample

Figure 4.2. Relationship between hydrocarbon concentrations in scallop adductor muscle and hydrocarbon concentrations in the exposure water.

Table 4.1. Accumulation of hydrocarbons in adductor muscle exposed for 24 h to condensate WSF.

Hydrocarbon Exposure Level (ppm)	Hydrocarbon Tissue Level (ppm)	Level of Significance of Taint	Total[1] Hydrocarbon Accumulation	Hydrocarbon Accumulation Factors[2]								
				toluene	m+p xylene	o-xylene	alkylated benzenes and others Peaks 44-53[3]	naphthalene	2-methyl-naphthalene	dimethyl-naphthalene and others Peaks 62-73	trimethyl-naphthalene and others Peaks 74-81	PNAH[4]
0.45	0.70	not significant	1.5	.5	.9	.2	.7	2.3	2.3	.7	5.6	0.5
0.78	1.38	not significant	1.8	.5	.9	1.5	1.9	3.7	5.3	8.1	9.1	0.9
2.66	15.59	p <.001	5.9	.4	.8	1.1	14.0	4.9	20.0	44.5	25.0	7.5
3.79	48.77	p <.001	12.9	.6	1.2	1.4	14.0	6.1	19.0	26.0	7.5	5.0

[1]Tissue level divided by exposure level.
[2]The ratio of the individual hydrocarbon concentration to the total hydrocarbon concentration in the adductor muscle divided by the equivalent ratio in the exposure water.
[3]See Figure 3.1 for identification of peak numbers.
[4]Polynuclear aromatic hydrocarbons.

higher exposure levels (2.66 to 3.79 ppm) for the same duration, there was a more significant accumulation of condensate WSF in the adductor muscle, in the order of 5.9 to 12.9 x the exposure level. These latter scallops were significantly tainted (Figure 4.3; Table 4.1). The correlation between the hydrocarbon level in the exposure water and in the adductor muscle was very strong. The data indicate a tainting threshold tissue level between 1.38 and 15.59 ppm (Figures 4.3 and 4.4). However, considering the degree of tainting in the more contaminated adductor muscle, the actual threshold concentration in the tissue is probably closer to 1.38 than to 15.59 ppm.

Table 4.1 provides a more detailed look at the individual hydrocarbon components which accumulated in the scallop adductor muscle. Although toluene and the xylenes were the most significant components in the exposed adductor muscle, the net accumulation of these components reflected their relative contribution to the total WSF, with an accumulation factor of about 1 (0.2 to 1.5). This indicates that there was no selective uptake or accumulation of toluene and xylenes. On the other hand, there were relatively higher proportions of the alkylated benzenes, naphthalene, and alkylated naphthalenes in exposed adductor muscle compared to the corresponding proportions in the exposure water.

This suggests that scallops either selectively take up these higher molecular weight aromatics from the exposure water and/or that these components are metabolized at a slower rate than toluene and xylene and other lower molecular weight aromatics. It should be noted that total naphthalenes have been shown to bioaccumulate in gall bladder bile by a factor of 1,000 or more in fish exposed to the WSF of fuel oil for a few days (Dixit and Anderson, 1977). Melzian and Lake (1987) observed a similar trend in blue crabs exposed to No. 2 fuel oil. These crabs showed retention of alkylated naphthalenes after other hydrocarbon components had depurated.

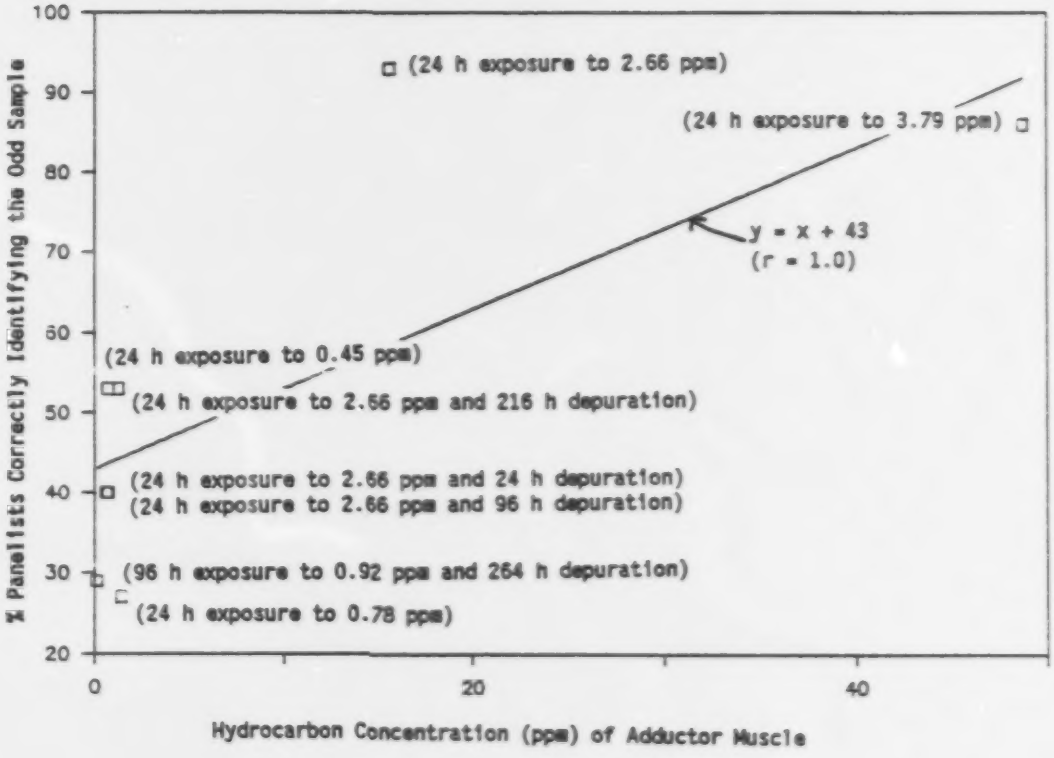
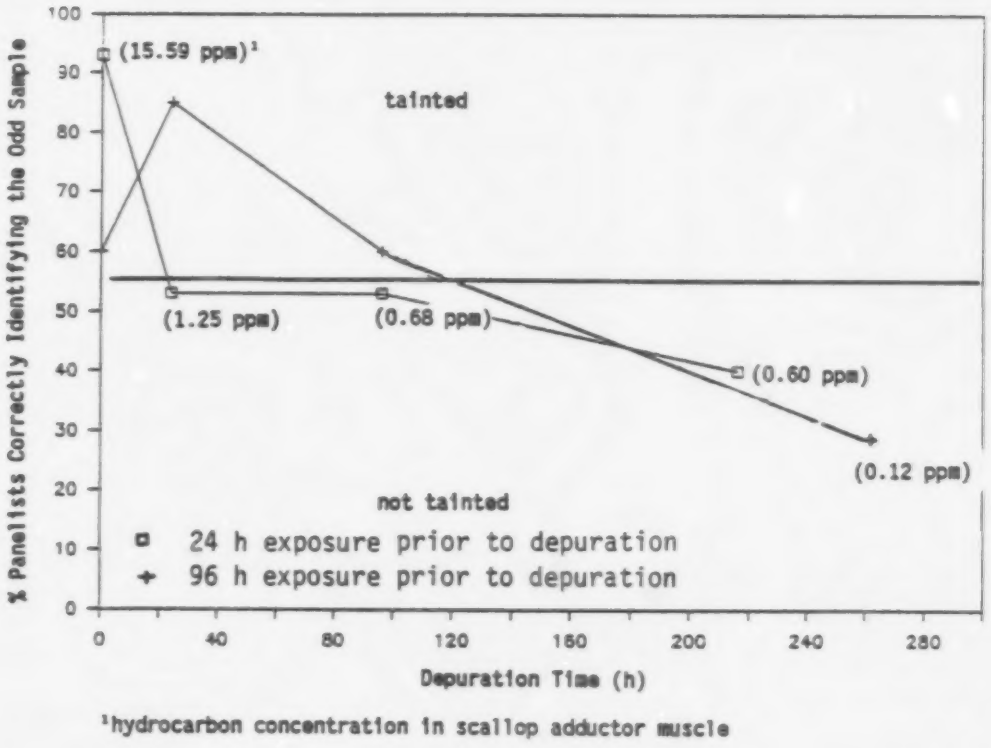

100
90
80
70
60
50
40
30
20
0
20
40
% Panelists Correctly Identifying the Odd Sample
Hydrocarbon Concentration (ppm) of Adductor Muscle
(24 h exposure to 2.66 ppm)
(24 h exposure to 3.79 ppm)
y = x + 43
(r = 1.0)
(24 h exposure to 0.45 ppm)
(24 h exposure to 2.66 ppm and 216 h depuration)
(24 h exposure to 2.66 ppm and 24 h depuration)
(24 h exposure to 2.66 ppm and 96 h depuration)
(96 h exposure to 0.92 ppm and 264 h depuration)
(24 h exposure to 0.78 ppm)

Figure 4.3. Relationship between taste panel results and hydrocarbon concentrations in the scallop adductor muscle.



[1]hydrocarbon concentration in scallop adductor muscle

Figure 4.4. Relationship between taste panel results and duration of depuration.

Melzian and Lake (1987), observing selective metabolism of lower molecular weight hydrocarbons in blue crabs, speculated that changes in the concentration and relative proportions of individual hydrocarbon components may be related to molecular weight and configuration, water solubility, and the enzyme or membrane specificity of the animal itself.

It was interesting to observe that the selective accumulation of the larger aromatic compounds was enhanced in those scallops exposed to fairly high levels of condensate WSF (2.66 and 3.79 ppm). For example, the accumulation of the larger aromatics noted in Table 4.1 was 2 to 7 x higher in these scallops compared to scallops exposed to relatively low levels of condensate WSF. The net effect of both the selective accumulation of larger aromatics and the enhancement of accumulation due to increasing the exposure level of WSF can be, for example, an accumulation factor of 44 for dimethyl-naphthalene compared to 0.5 for toluene.

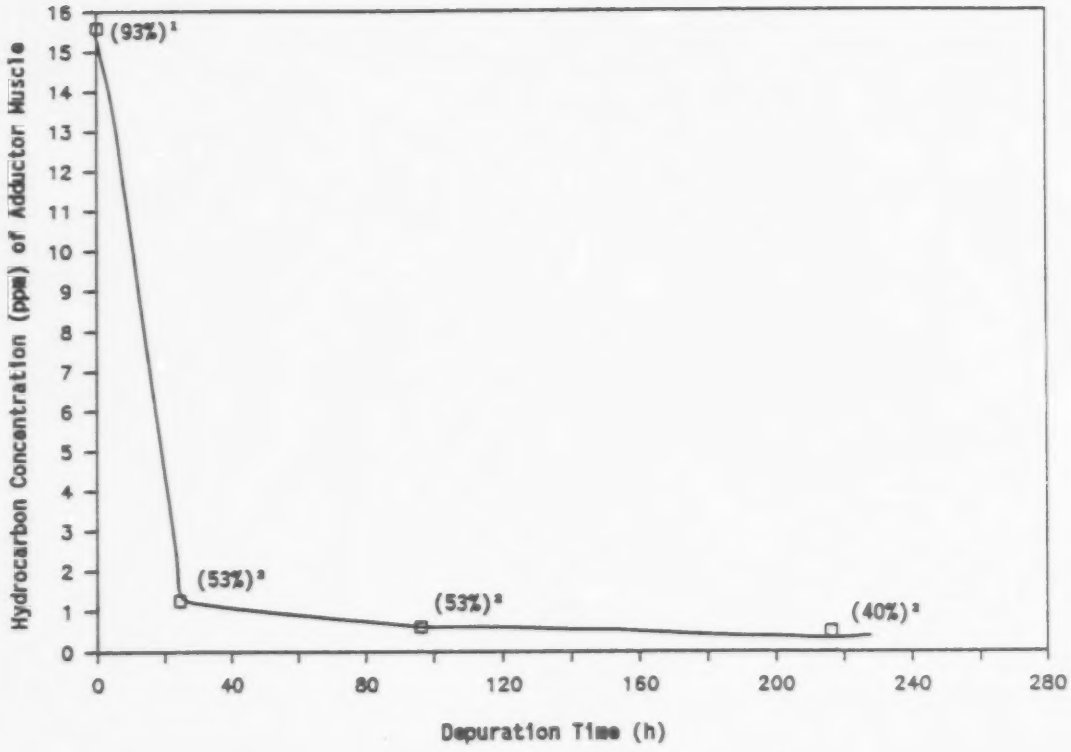
The intensity of taint in the edible tissue of fish and shellfish not only differs with the total hydrocarbon content, but also with the types, number of carbon atoms, and molecular structure (Motohiro, 1983; Motohiro and Iseya, 1976; Mackie et al., 1972; Ogata et al., 1987, Ogata and Miyake, 1973; Ogata and Ogura, 1976). Saturated hydrocarbons are not important in influencing the flavour of scallops or other marine animals, but both unsaturated aliphatics and aromatic hydrocarbons impart strong off-flavours in fish tissues (Motohiro and Iseya, 1976). The off-flavours from aromatics are more intense than those from unsaturated aliphatics. Among the aromatics, toluene and xylenes have been suggested as the main components responsible for tainting (Motohiro, 1983; Ogata and Miyake, 1973; Ogata and Ogura, 1976). In our study, tainted scallops had very high levels of toluene and xylene. These two hydrocarbons were not detected in untainted tissue, whereas larger aromatics such as the alkylated benzenes and naphthalenes were present, although at low levels. This suggests that the taint in scallops is mainly due to uptake of toluene and xylene from the WSF of condensate. Alkylated benzenes,

naphthalene, and alkylated naphthalenes could contribute to tainting when they are present at relatively high concentrations, but do not cause tainting when retained in the adductor muscle at relatively low levels.

4.3.4 Depuration

The results of the depuration trials involving 24 h exposures are summarized in Figures 4.4 and 4.5 and Table 4.2. These indicate that even with short-term exposure to relatively high concentrations of condensate WSF, depuration of 24 h or less results in a loss of taint. This was apparently related to a rapid reduction in the total hydrocarbon burden in the adductor muscle, which was mostly due to the loss of the light aromatic compounds such as toluene and xylenes (Table 4.2). On the other hand, longer exposure to relatively low levels of condensate WSF resulted in a more stable taint, which was only lost between 4 and 11 days into depuration. This suggests that more chronic low level exposure to hydrocarbons results in a different metabolic processing of some of the accumulated hydrocarbons, as has been noted in other studies (see Power, 1980 for a review).

Table 4.2 indicates that after the first 24 hours of depuration, only 8% of the original hydrocarbon accumulation remained in the adductor muscle. Most of the loss was due to the very rapid depuration of the lighter aromatics such as toluene, xylenes, and alkylated benzenes. On the other hand, Table 4.2 clearly shows the selective retention of trimethylnaphthalenes and other polynuclear aromatic hydrocarbons. The same factors which contribute to the selective accumulation of these larger aromatic compounds must also be reflected in how fast they are depurated from the tissue. It is interesting to note that despite the apparent retention of the larger aromatic compounds, the adductor muscles were not tainted, further implicating the light aromatic components as the tainting compounds.



¹% panelists correctly identifying the odd sample (tainted)

²% panelists correctly identifying the odd sample (not tainted)

Figure 4.5. Relationship between hydrocarbon concentration in scallop adductor muscle and duration of depuration (scallops exposed for 24 h to 2.66 ppm hydrocarbon).

Table 4.2. Retention of hydrocarbons in adductor muscle which had been exposed for 24 h to 2.66 ppm hydrocarbon followed by a depuration phase - initial adductor muscle hydrocarbon concentration 15.59 ppm.

Depuration Time (h)	Level of Significance of Taint	Total[1] Hydrocarbon Retention	Hydrocarbon Retention Factors[2]								
			toluene	m+p xylene	o-xylene	alkylated benzenes and others Peaks 44-53[3]	naphthalene	2-methyl-naphthalene	dimethyl-naphthalene and others Peaks 62-73	trimethyl-naphthalene and others Peaks 74-81	PNAH[4]
24	not significant	0.08	.07	.3	.3	.6	.6	.6	.7	5.1	5.3
96	not significant	0.04	.07	.1	.3	.5	.3	.2	1.2	3.8	5.9
216	not significant	0.04	.08	.1	.3	2.1	.3	.2	1.2	6.7	6.7

[1]Hydrocarbon level in depurated adductor muscle divided by hydrocarbon level in adductor muscle which was not depurated.
[2]The ratio of the individual hydrocarbon concentration to the total hydrocarbon concentration in the depurated adductor muscle divided by the equivalent ratio at time 0 (not depurated).
[3]See Figure 3.1 for identification of peak numbers.
[4]Polynuclear aromatic hydrocarbons.

4.4 Implications for Gas Field Development on the Scotian Shelf

The results from the present study indicate that the experimental set-up has permitted very detailed resolution of the threshold level of condensate WSF required to cause tainting in sea scallops and that the exposure conditions can be repeated accurately with reliable results. However, these results are only useful if they can be extrapolated to a field situation in which there may be environmental concerns.

The difficulty in extrapolating laboratory data on tainting to a real spill or blowout situation lies in the comparison of the WSF responsible for tainting in the laboratory with what is usually reported as whole oil or condensate under a slick. This problem can be dealt with in an iterative way by first of all examining the percentage of whole condensate that remains as a true WSF, calculating the concentration of whole condensate which must be sustained in the water column to produce a tainting threshold of WSF, and then comparing the required concentration of whole condensate to concentrations that have been observed or calculated in the past.

The first step involves an analysis of the mixing ratios (condensate:water) and the resultant concentration of the WSF. Three mixing ratios were analyzed (see Appendix A). For mixing ratios ranging from 1:99 to 1:299, the percentage of the original whole condensate volume that remained in the water as WSF ranged from 0.9% to 1.7%. There was clear evidence that reducing the ratio (decreasing the volume of condensate in a given volume of seawater) resulted in a larger percentage of the condensate volume remaining as WSF. However, the log normal relationship between the mixing ratio and the percentage of condensate that is dissolved ($x = -2.4987 + 0.7430 \ln y$; $r = 0.99$, where x is the percentage dissolved and y is the mixing ratio) also indicates that there is a levelling off of the percentage dissolved, such that even for very

small mixing ratios which reflect a real blowout situation (1:10,000 to 1:1,000,000) the percentage dissolved ranges between 4% and 8% of the original volume of whole condensate. For the purpose of analysis, we can assume that no more than 8% of the original whole condensate volume remains in the water column as a true WSF such as that made in the laboratory. With a tainting threshold of 0.8 ppm (condensate WSF), and assuming that this does not exceed 8% of the original condensate volume as noted above, the concentration of whole condensate which must be sustained in the water column for at least 3 h exceeds 10 ppm.

According to studies by Ross (1982), the maximum initial concentration of whole condensate during a blowout would not exceed 8.5 ppm. The concentration is rapidly reduced over time (with no further input of condensate) and away from the location of the blowout. Even this value, which is based on some conservative assumptions, may be higher than normally expected. According to McAuliffe (1986), sustained whole oil-in-water concentrations under naturally dispersed oil slicks are usually less than 1 ppm. During the Uniacke blowout, in which natural gas condensate entered the water from the air (with much initial loss of the lighter compounds below C_{10}), there were few water samples with condensate concentrations exceeding 300 ppb (Carter *et al.*, 1985). These observations are consistent with observations of light oil at other spills and with the large initial evaporative loss expected in such oils.

It can be concluded from the analysis above that the condensate WSF tainting threshold is unlikely to be achieved in the water column, especially near the seabed, during a short-term blowout. Thus, for sea scallops on the seabed, the risk of tainting due to a short-term condensate blowout is low. The risk of toxic effects in adult scallops is even lower.

Other factors not examined in this study could alter this conclusion. For example, if there are other components in the condensate, other than the WSF, which contribute to tainting, the risk could

increase. Similarly, long-term exposure (greater than 96 h) to dissolved condensate at concentrations lower than the levels examined in this study could result in tainting as well.

4.5 Possibilities for Future Tainting Research

A comparison of the results of this study and results from earlier work with scallops exposed to the WSF of Ninian crude oil indicates similarity in the composition of the two WSFs and a very similar exposure level/tainting relationship. This suggests that further work on different WSFs may not be warranted. Future tainting research should focus on other variables which have not been examined in a definitive way in the past. These include the following:

1. The effect of lipid content in the exposed animal on the degree of tainting. Tainting research in this region has focussed on low-lipid animals such as cod and scallops. Tainting research on flatfish and oily fish such as salmonids would be useful.

2. The tainting potential of individual compounds in the WSF (such as toluene, xylene, naphthalene, mercaptans, etc.) should be determined. This could be examined on a single and additive basis to establish whether there are synergistic effects due to the presence of more than one tainting compound.

3. The degree of tainting in scallops exposed over the long-term (> 96 h) to low levels of WSF should be examined, since this may approximate some blowout scenarios (Uniacke - 10 days; Ixtoc - in the order of months).

In addition to the three main priorities noted above, it might also be useful: to examine tainting in scallop roe, since the roe may be exported for consumption elsewhere; examine tainting in scallops exposed

to an oil-in-water dispersion; examine the effect of different mixing ratios on the percentage of the whole oil or condensate that dissolves, since this information is important in extrapolating laboratory data to a field situation; and, examine the pros and cons of different exposure and taint evaluation methodologies, to improve the interpretation of data from different studies.

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Mr. John Carter (Martec Limited)	- Project Manager, Data Interpretation, Editing
Ms. Rosslyn Ernst (Martec Limited)	- Exposure System Design and Monitoring
Dr. Nimal Ratnayake (CIFT)	- Gas Chromatography
Ms. Terri Farquharson (CIFT)	- Taste Panel Evaluation
Dr. Bob Gershey (Seakem Oceanography Limited)	- Toxicity Testing
Mr. Remi Odense (Seakem Oceanography Limited)	- Toxicity Testing

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APPENDICES

APPENDIX A

RESOLUTION OF WSF MIXING RATIO

The water-soluble fraction (WSF) refers to that fraction of oil or condensate which dissolves in water. Different laboratories have used a variety of methods to prepare WSFs, with ratios of oil to water ranging from 1:6 to 1:1,000 (Murray et al., 1984). The wide range of oil to water ratios reported by others has produced a range of concentrations of hydrocarbons in the WSF. Murray et al. (1984) have reported a concentration range of 1.7 to 72.1 ppm hydrocarbon for many crude oils and petroleum products.

The concentration of hydrocarbons in the WSF depends on the:

- type of hydrocarbons;
- ratio of hydrocarbon and water;
- rate at which the hydrocarbon and water are agitated or mixed;
- duration of mixing; and,
- settling time required to achieve a stable distribution of hydrocarbons between the aqueous and oil phases.

Lockhart et al. (1984) examined in detail the above parameters and concluded that an oil to water ratio of about 1:100 produced a WSF saturated with hydrocarbons for many types of oils. Using high quantities of oil yields a WSF with a higher concentration of hydrocarbons, but the yields reported are low in comparison with the increased amount of oil used. Lockhart et al. (1984) have generally recommended a mixing time of 24 h with a settling time of 48 h to attain

equilibrium or a stable distribution of hydrocarbons. The method of mixing seems to matter relatively little with any type of mechanical stirring or shaking producing a suitable WSF.

In previous studies (Ernst et al., 1988 a,b), a mixing ratio of 1:99 was used in preparing WSFs of a variety of crude oils. Because of the small volume of condensate available for this study, we investigated, on a laboratory scale, the suitability of adopting an even lower ratio of condensate to water.

The WSF was prepared in the laboratory by adding known amounts of condensate and seawater to a 6 L Erlenmeyer flask and mixing with a magnetic stirring bar for 24 h in a 5°C cold room. The top of the flask was covered with aluminum foil and then sealed with parafilm. The whole mixture was transferred to a 6 L separatory funnel and allowed to settle for 48 h at 5°C. At the end of the settling period, a 980 mL sample of the WSF (bottom layer) was withdrawn into a microextraction flask and the hydrocarbons in the WSF were extracted and analyzed by GC as described in Section 2.5.

The concentrations of hydrocarbons in the WSF prepared on the laboratory scale are noted below. Both the condensate to water ratios of 1:99 and 1:199 essentially saturated the water at a level two to three times that observed for crude oils prepared in the same manner (Ernst et al., 1988b). The batch prepared at a ratio of 1:99 was also analyzed after settling for 14 days and the results indicated only a 23% loss (70.83 ppm remaining).

Hydrocarbon Content of WSF
Prepared on Laboratory Scale

Condensate/Water	Hydrocarbon (ppm)
1:99	92.28
1:199	73.47

Given the high levels of hydrocarbons in the WSF prepared at ratios of 1:99 and 1:199 and the relative stability of the WSF over a 14 day period, it was decided to mix the large scale batches at an even lower ratio of 1:299 to conserve the volume of condensate available.

For the purpose of analysis of mixing ratios in Part 4, the relationship between the mixing ratio (condensate:water) and the percentage of the original condensate that remains dissolved was expressed as a long normal equation and plotted (Figure A.1). The dissolved fraction was predicted for small mixing ratios of 1:1,000 and 1:10,000.

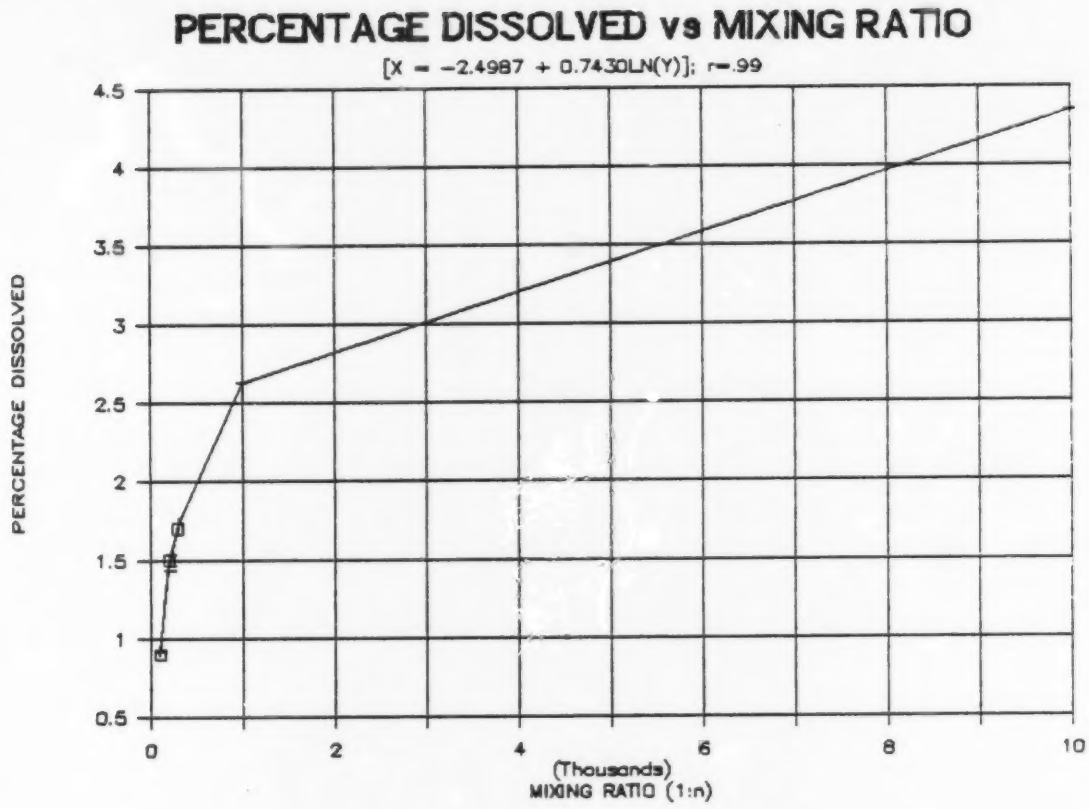

Figure A.1. Relationship between the mixing ratio and the percentage of original condensate that remains as a WSF.

APPENDIX B

STABILITY OF THE EXPOSURE SYSTEM

Two mock trials (without scallops present) were closely monitored to determine the stability of hydrocarbon content of the exposure water. During the first trial, the hydrocarbon content of the exposure water was monitored by fluorescence for 67 hours with four GC samples taken to check the fluorometric results. The trial was repeated with a second batch of WSF and the hydrocarbon content monitored by fluorescence alone over a 48 h period.

The results of both these trials are presented in Figure B.1. The hydrocarbon content is expressed as the fluorometric data which have been calibrated with GC analysis of water samples (see Appendix D). The results of GC analysis of water samples taken during the first, 67 h, trial are summarized in Table B.1 below and the GLC hydrocarbon profiles are shown in Figures B.2 to B.5.

Table B.1. Hydrocarbon concentration of exposure water during mock trial (67 h).

Time (h)	Hydrocarbon (GC) (ppm)
0	0.83
24	0.61
48	0.79
67	1.02

The almost 50% drop in hydrocarbon content indicated by the fluorometric data was the result of a decrease in the flow of WSF being delivered to the tank during the night. The flow was increased to the original rate and the fluorescence values correspondingly increased. The flow rates were more constant during the remainder of the trials as reflected in the hydrocarbon content of the exposure water.

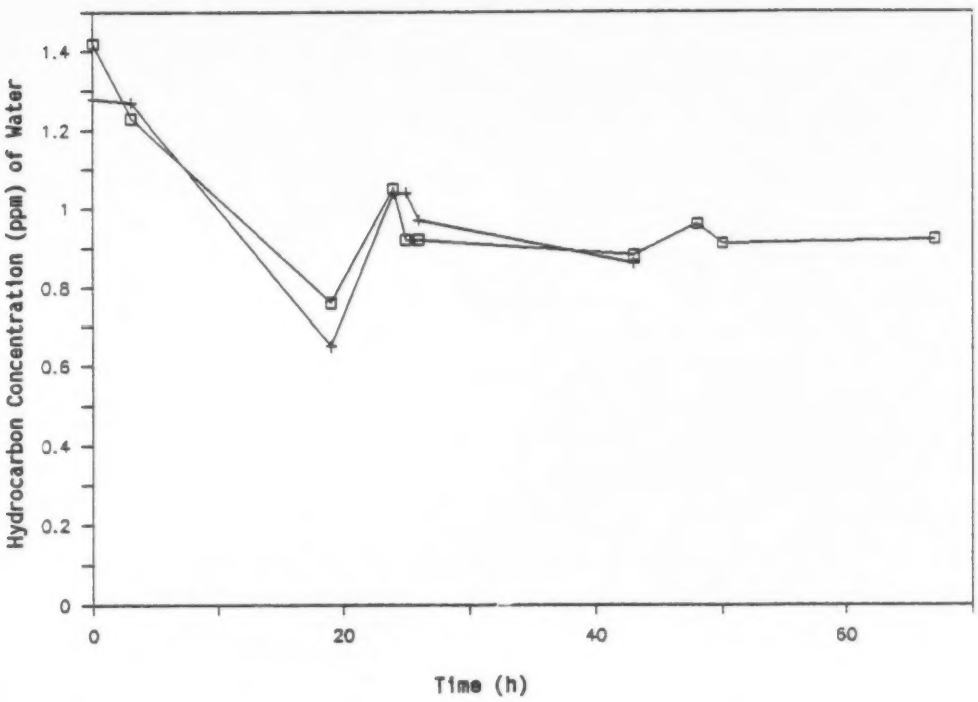

Figure B.1. Hydrocarbon concentrations in exposure water during two mock trials (no scallops present).

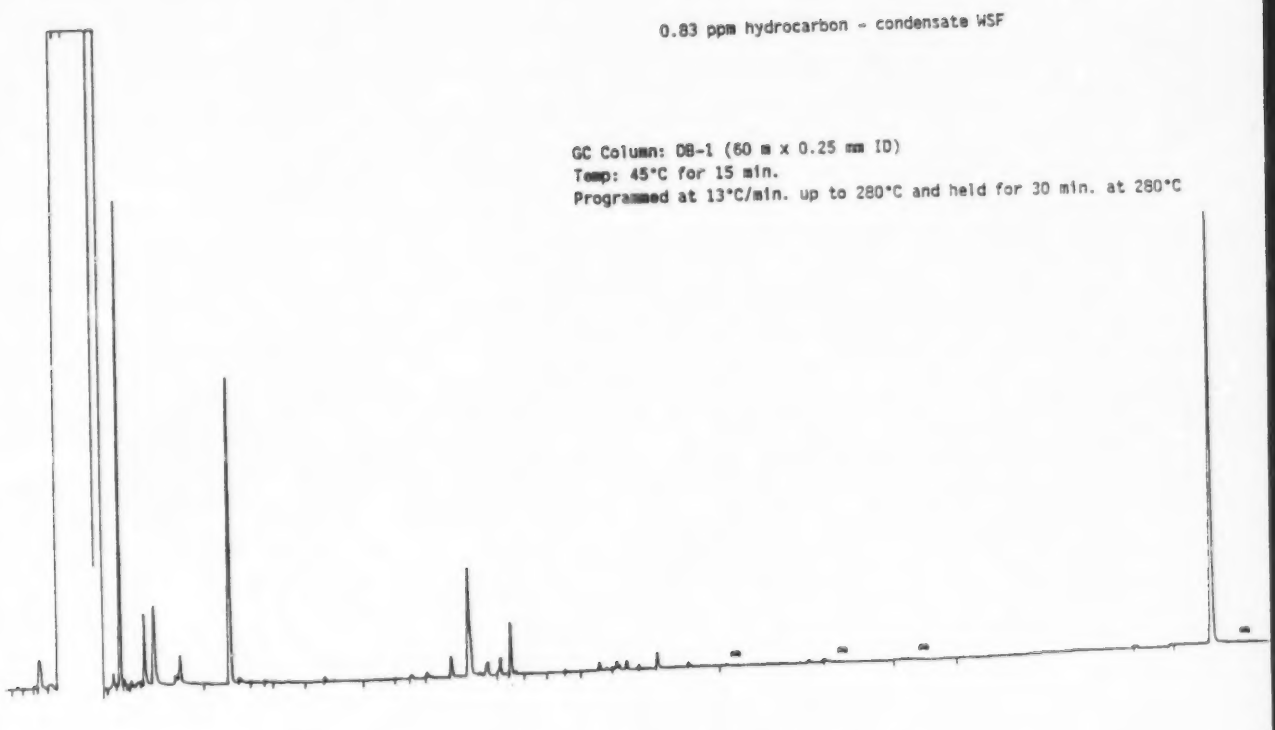
0.83 ppm hydrocarbon - condensate WSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and held for 30 min. at 280°C

Figure B.2. GLC hydrocarbon profile at time 0 from the 67 h mock exposure trial.

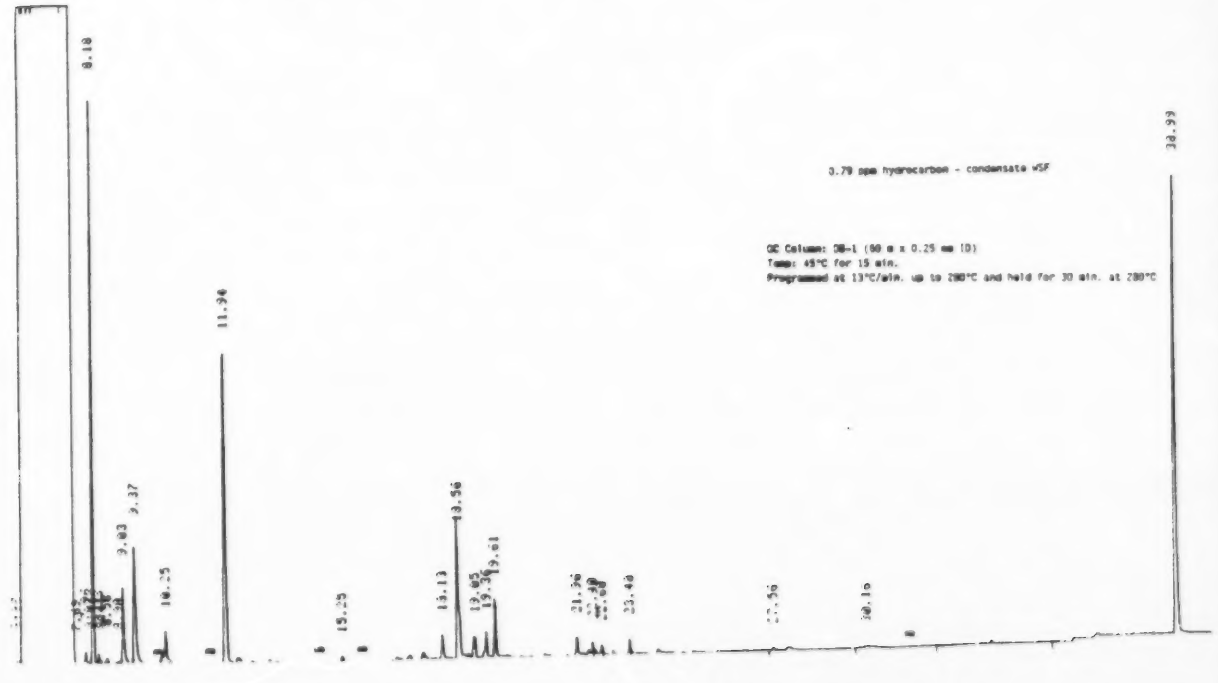
8.10
11.94
38.99
3.79 ppm hydrocarbon - condensate WSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and held for 30 min. at 280°C

Figure B.3. GLC hydrocarbon profile at time 24 h from the 67 h mock exposure trial.

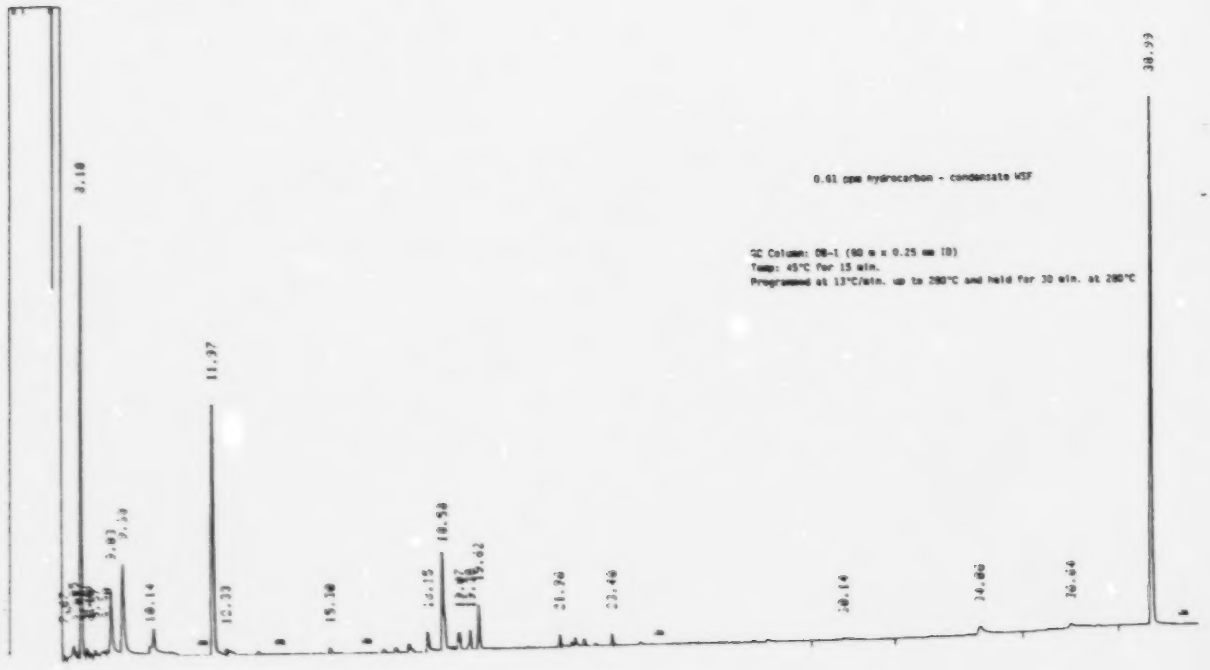
0.01 ppm hydrocarbon - condensate MSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and held for 30 min. at 280°C
38.99
11.97
10.58

Figure B.4. GLC hydrocarbon profile at time 48 h from the 67 h mock exposure trial.

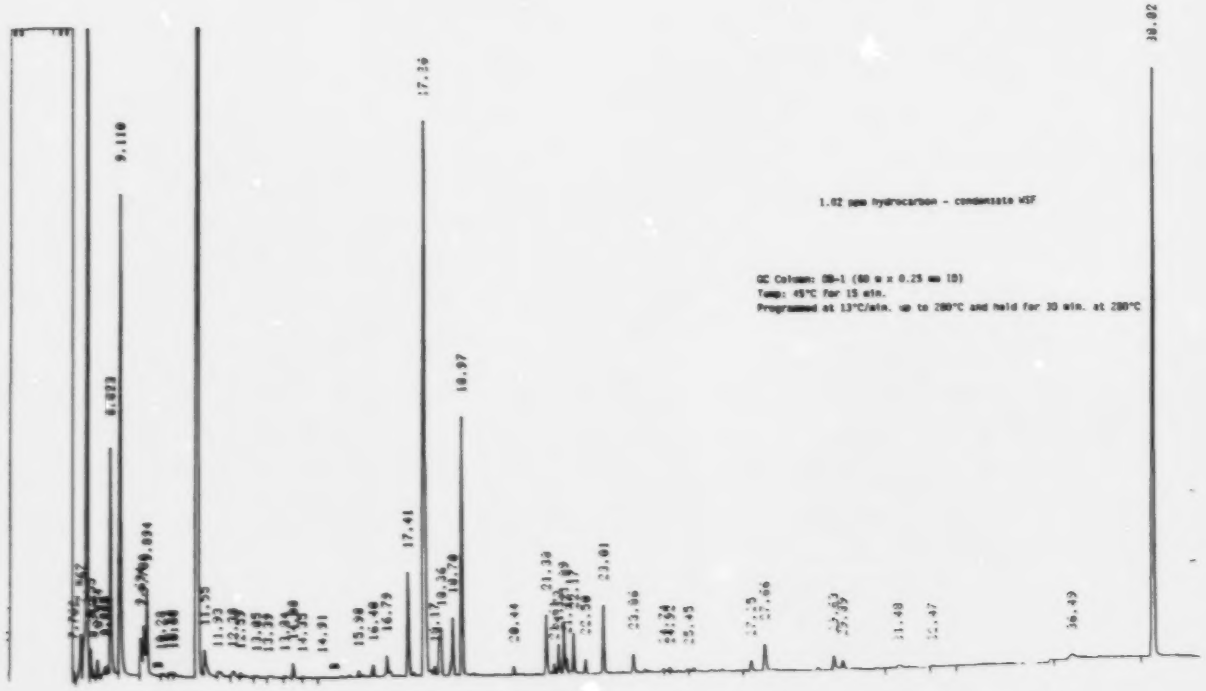
1.02 ppm hydrocarbon - condensate MSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and held for 30 min. at 280°C
38.82
17.38
9.110
10.97
17.41

Figure B.5. GLC hydrocarbon profile at time 67 h from the 67 h mock exposure trial.

The stability of the stock WSF held in the mixing vessel was also monitored at this time. The results of these analyses are presented in Table B.2 and the GLC hydrocarbon profiles are shown in Figures B.6 to B.9. A 25% loss in hydrocarbons was observed over the 3 days (48 to 120 h) that the system was monitored. During this time, WSF was being siphoned off for the toxicity trials as well as the mock tainting trial and, as such, represents the degree of loss expected during a prolonged exposure trial. The head space in the mixing vessel increased over time as WSF was being removed, thus increasing the potential for losses through evaporation.

Table B.2. Hydrocarbon concentration of stock WSF.

Hours Settled	Hydrocarbon (GC) (ppm)
48	52.69
72	60.23
96	40.74
120	39.03

It was concluded that the proposed exposure system would be adequate to maintain the desired levels of hydrocarbon in subsequent exposure trials, provided that the flow rates were closely monitored and adjusted if a marked increase or decrease was observed.

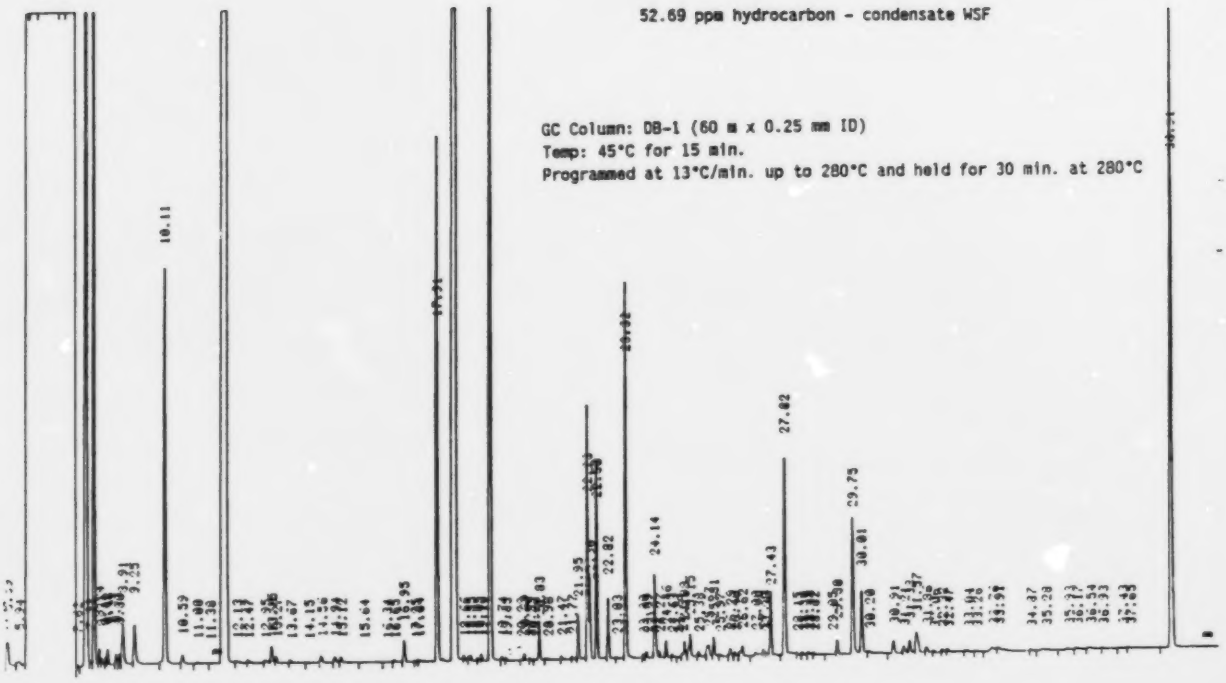
52.69 ppm hydrocarbon - condensate WSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and held for 30 min. at 280°C

Figure B.6. GLC hydrocarbon profile of stock WSF settled for 48 h.

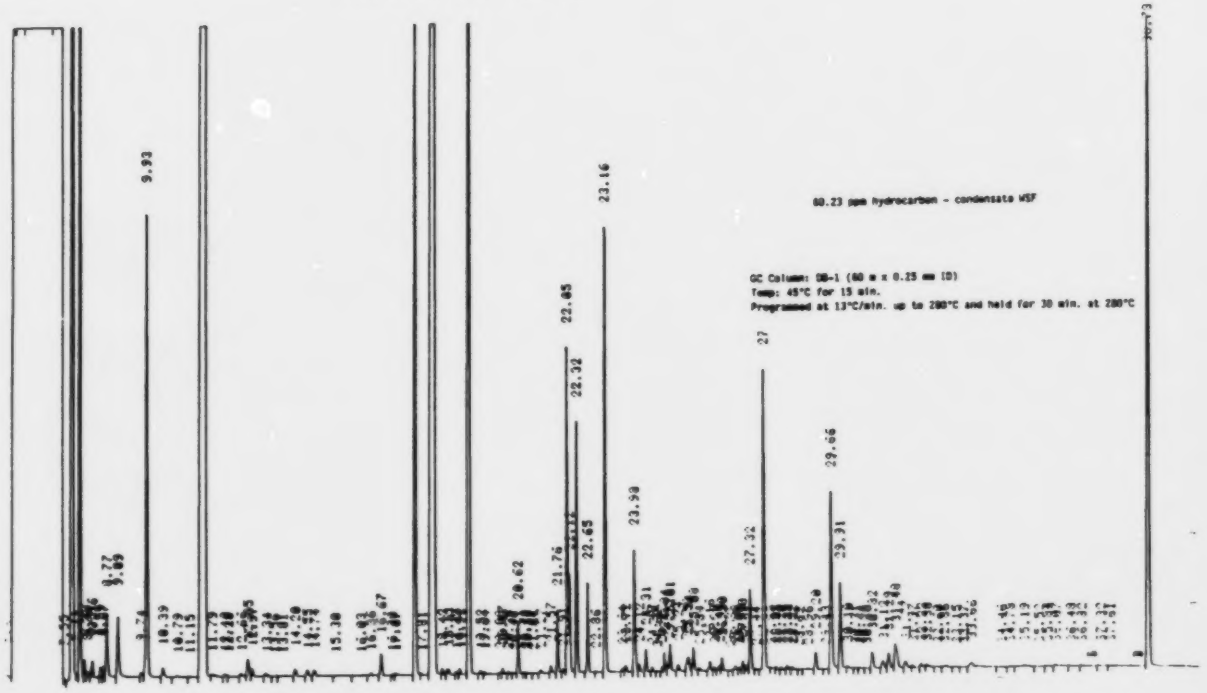
80.23 ppm hydrocarbon - condensate WSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and held for 30 min. at 280°C

Figure B.7. GLC hydrocarbon profile of stock WSF settled for 72 h.

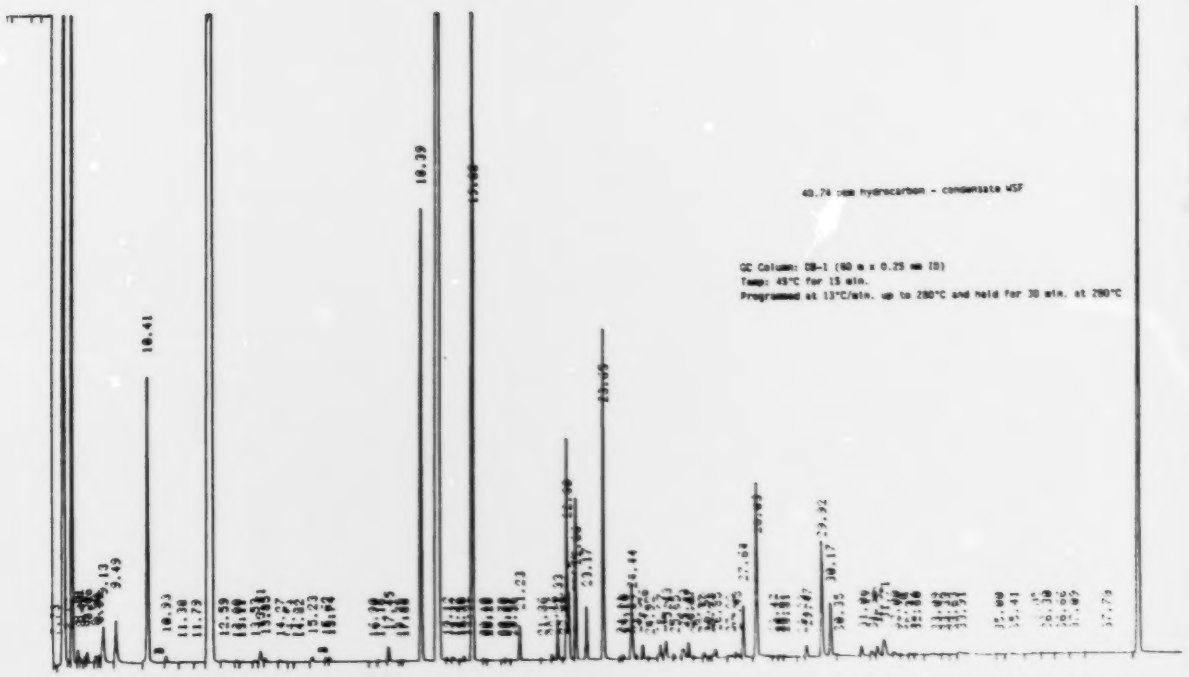

Figure B.8. GLC hydrocarbon profile of stock WSF settled for 96 h.

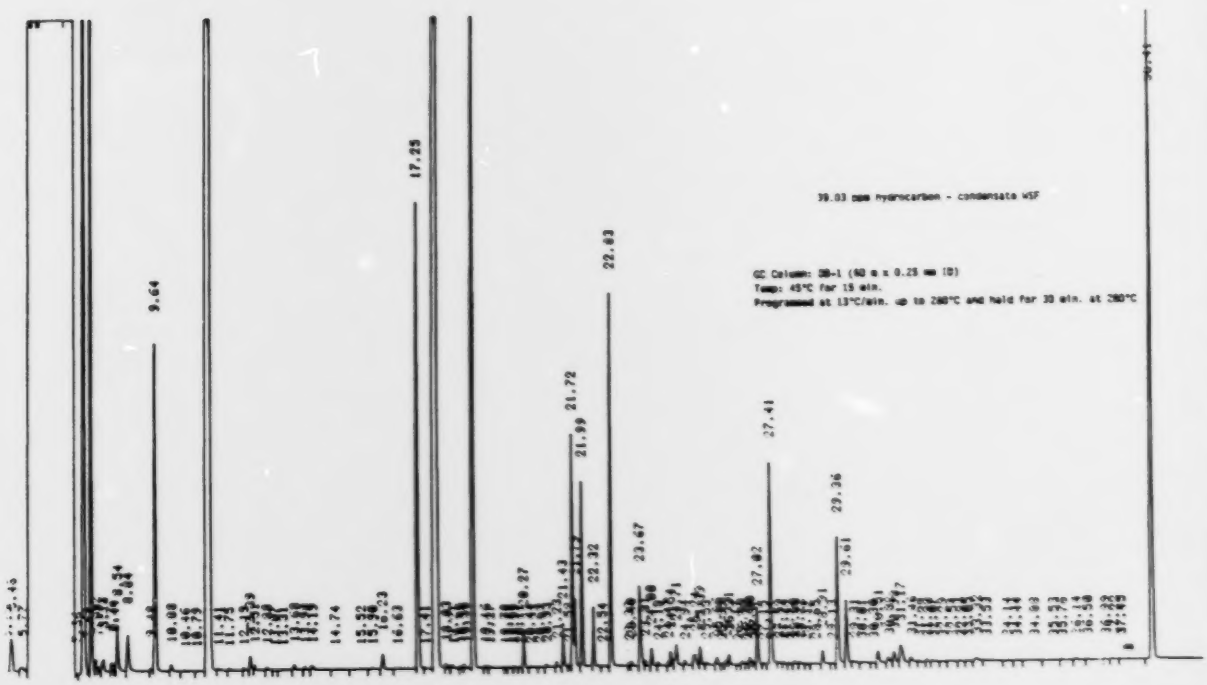

Figure B.9. GLC hydrocarbon profile of stock WSF settled for 120 h.

APPENDIX C

GLC HYDROCARBON PROFILES OF EXPOSURE WATER FROM THE TAINTING TRIALS

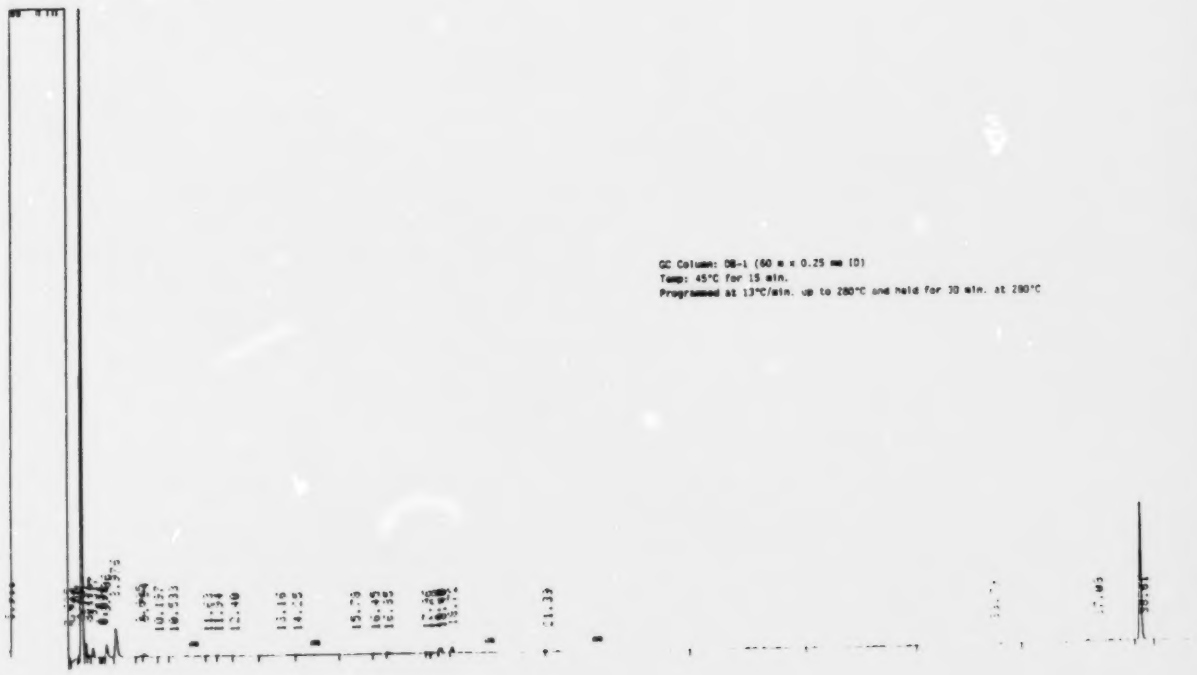

Figure C.1. GLC hydrocarbon profile of diluent seawater.

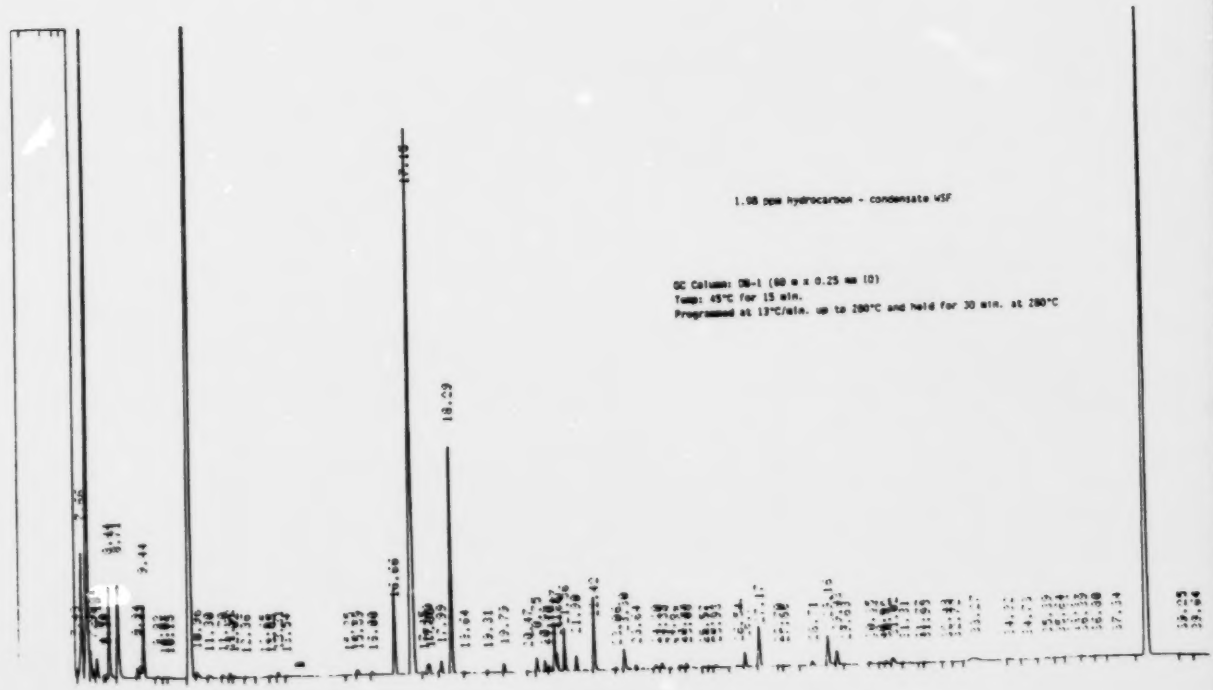
1.98 ppm hydrocarbon - condensate WSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and held for 30 min. at 280°C

Figure C.2. GLC hydrocarbon profile at time 0 from the 3 h 2.10 ppm exposure.

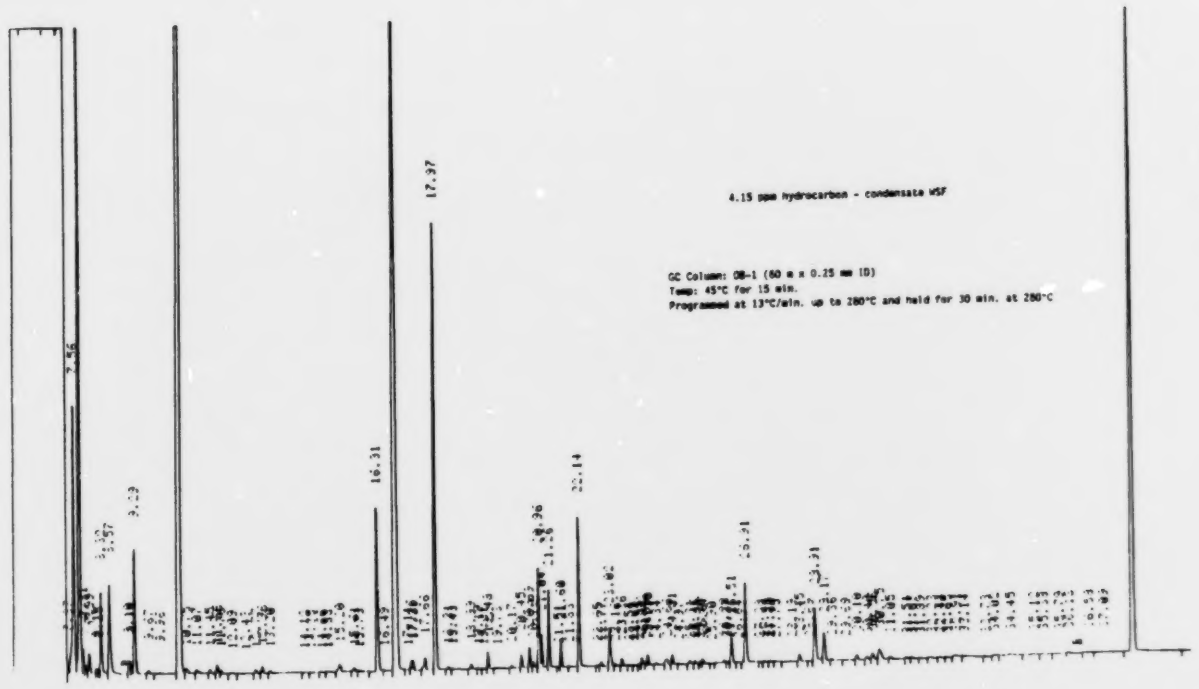
4.15 ppm hydrocarbon - condensate WSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and held for 30 min. at 280°C
17.37
16.31
22.14

Figure C.3. GLC hydrocarbon profile at time 0 from the 3 h 3.55 ppm exposure.

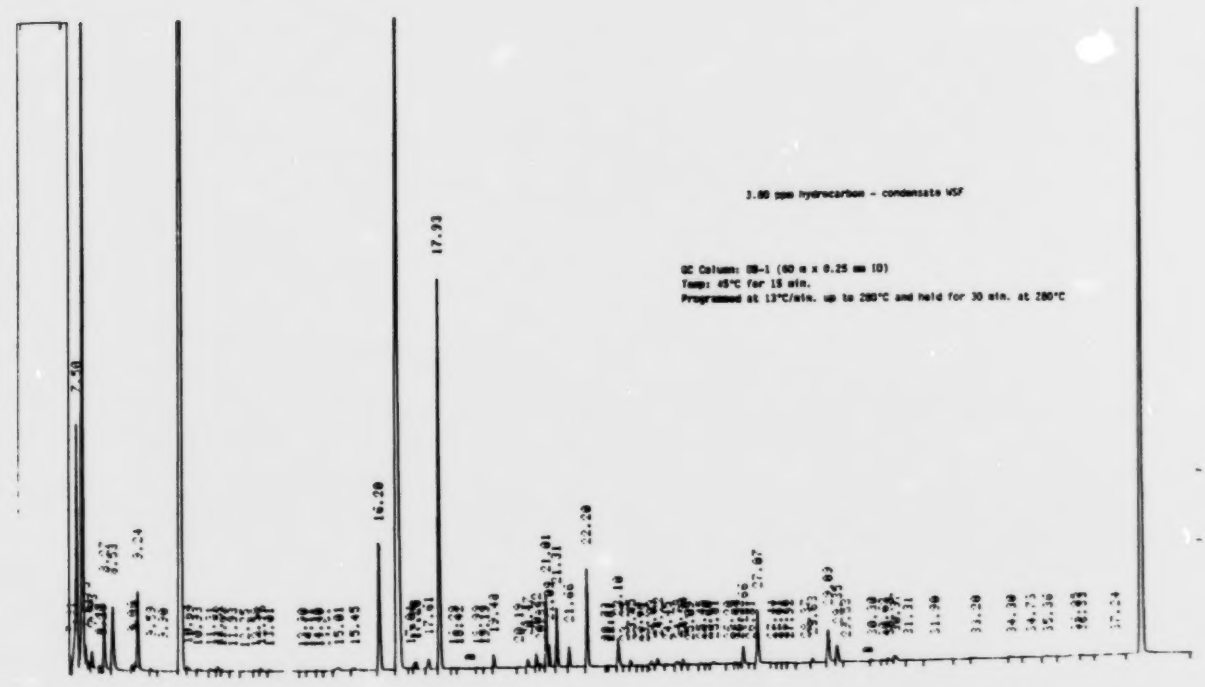
3.80 ppm hydrocarbon - condensate WSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and held for 30 min. at 280°C
17.93
16.20
22.20

Figure C.4. GLC hydrocarbon profile at time 3 h from the 3 h 3.55 ppm exposure.

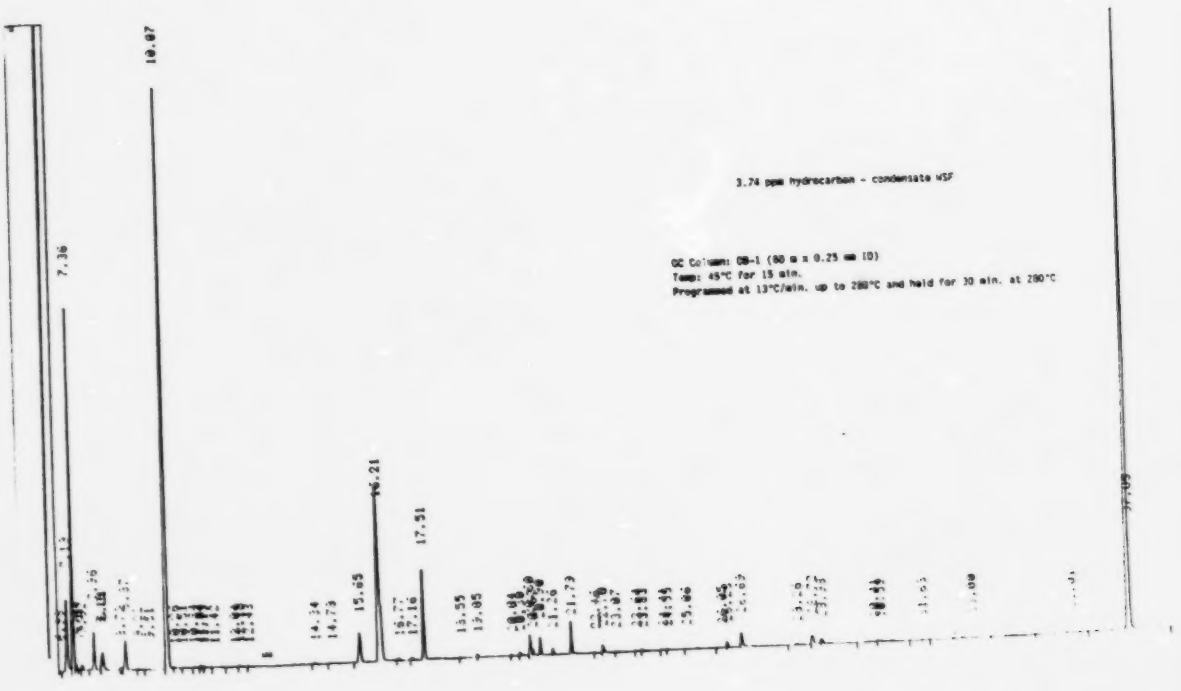
10.07
7.36
16.21
17.51
3.74 ppm hydrocarbon - condensate WSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and held for 30 min. at 280°C

Figure C.5. GLC hydrocarbon profile at time 0 from the 3 h 4.76 ppm exposure.

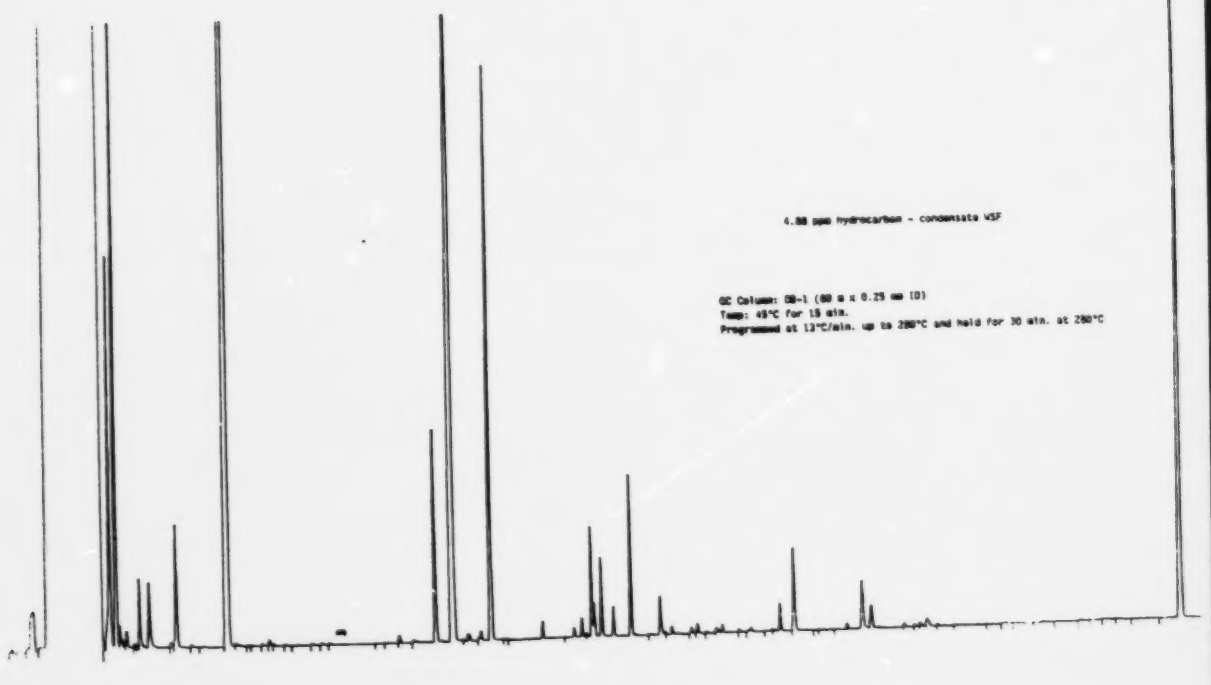
4.88 ppm hydrocarbon - condensate WSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and held for 30 min. at 280°C

Figure C.6. GLC hydrocarbon profile at time 3 h from the 3 h 4.76 ppm exposure.

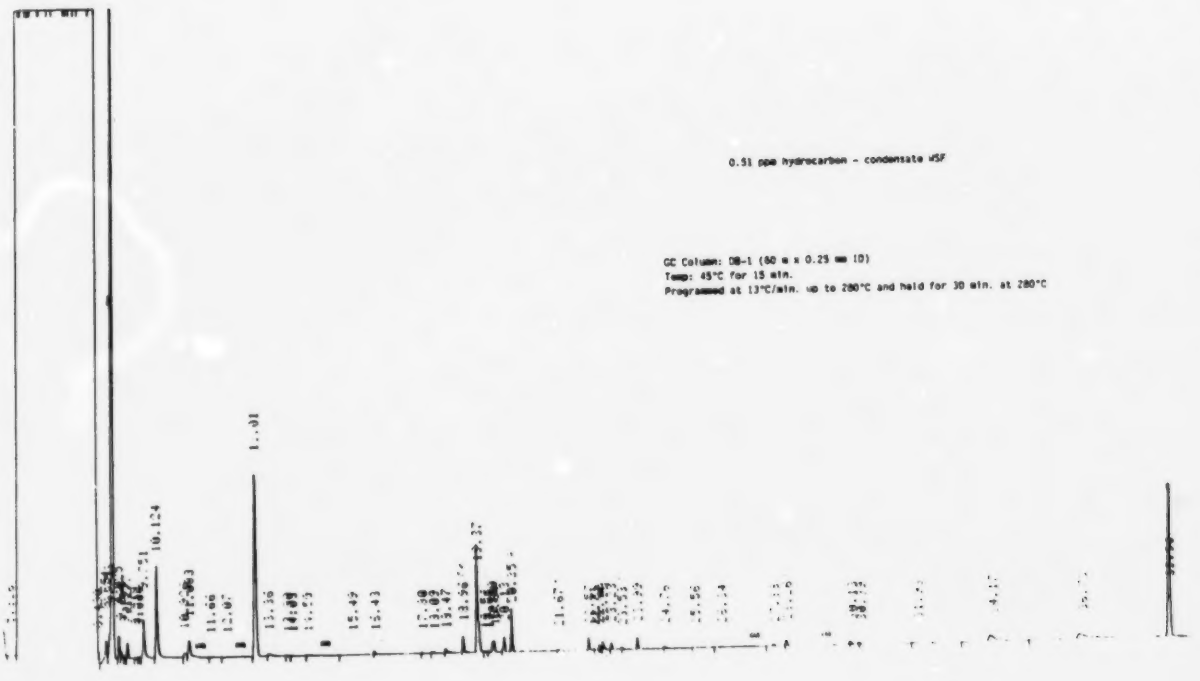
0.51 ppm hydrocarbon - condensate WSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and held for 30 min. at 280°C

Figure C.7. GLC hydrocarbon profile at time 0 from the 24 h 0.45 ppm exposure.

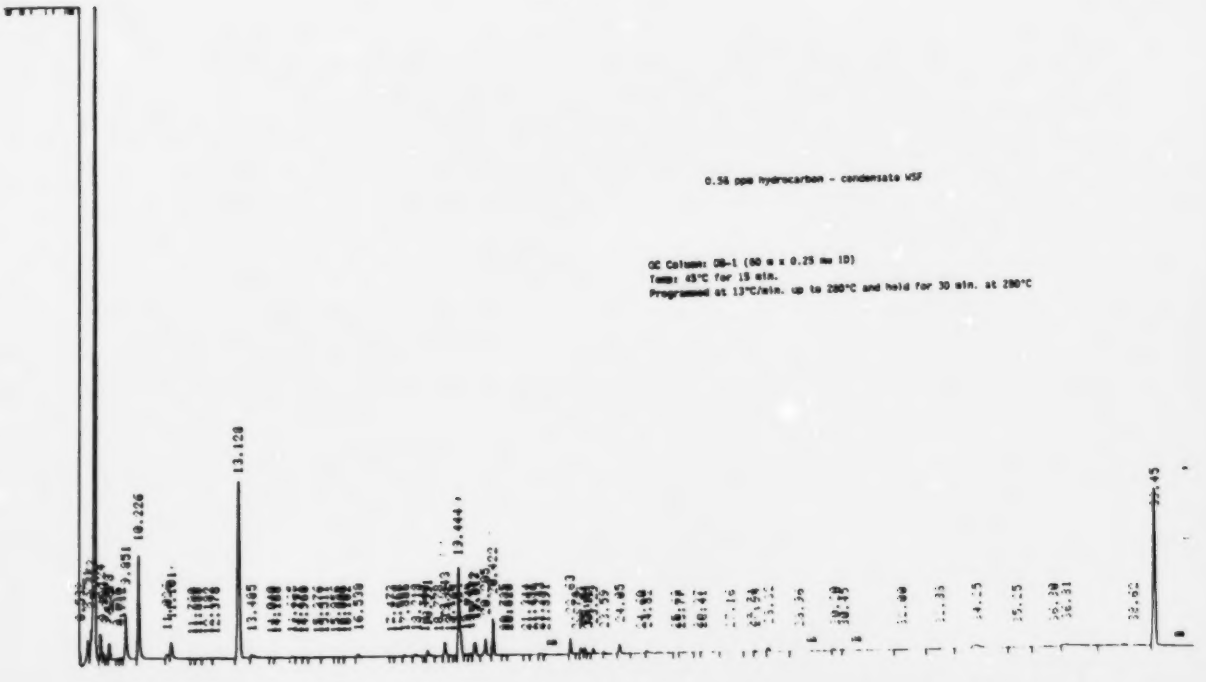
0.56 ppm hydrocarbon - condensate WSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and held for 30 min. at 280°C

Figure C.8. GLC hydrocarbon profile at time 24 h from the 24 h 0.45 ppm exposure.

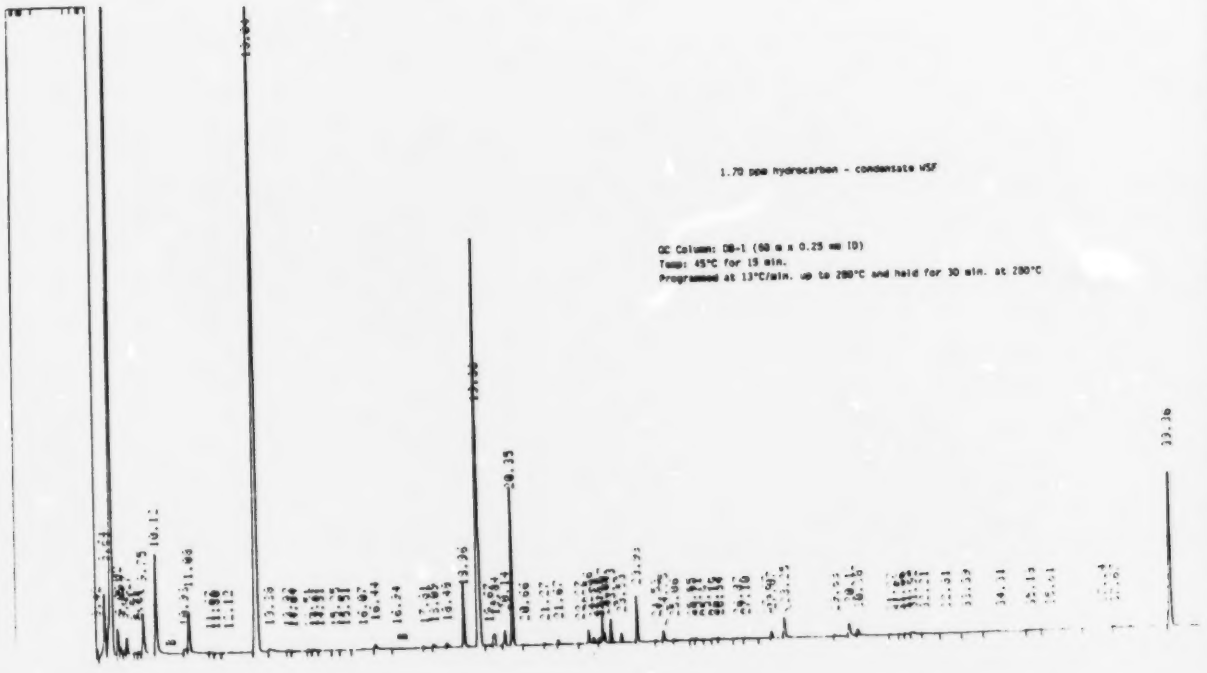
1.70 ppm hydrocarbon - condensate WSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and held for 30 min. at 280°C

Figure C.9. GLC hydrocarbon profile at time 0 from the 24 h 0.78 ppm exposure.

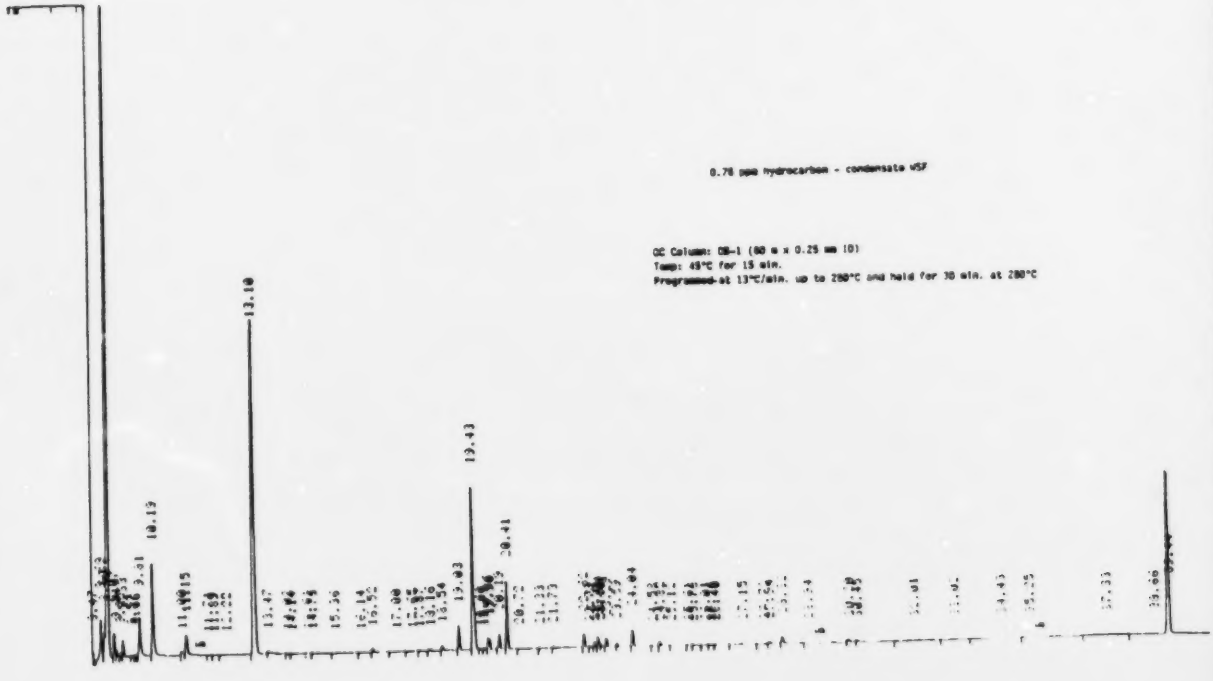
0.78 ppm hydrocarbon - condensate WSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and held for 30 min. at 280°C

Figure C.10. GLC hydrocarbon profile at time 24 h from the 24 h 0.78 ppm exposure.

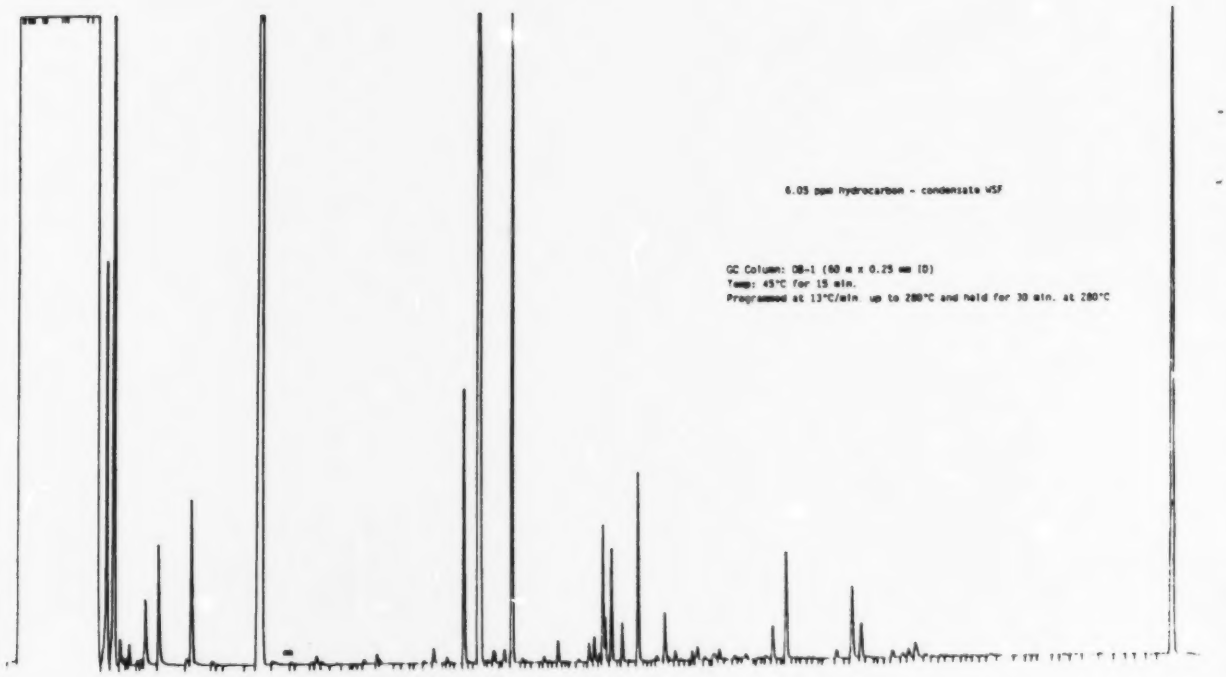
6.05 ppm hydrocarbon - condensate WSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and held for 30 min. at 280°C

Figure C.11. GLC hydrocarbon profile at time 0 from the 24 h 3.79 ppm exposure.

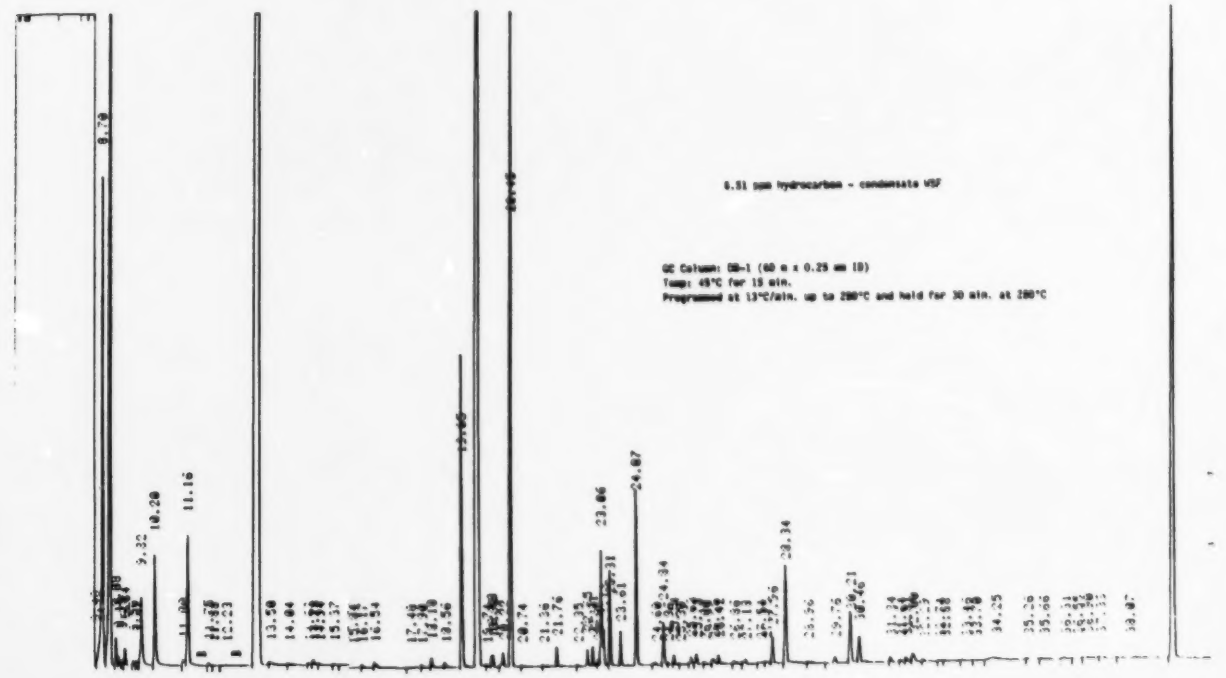

Figure C.12. GLC hydrocarbon profile at time 24 h from the 24 h 3.79 ppm exposure.

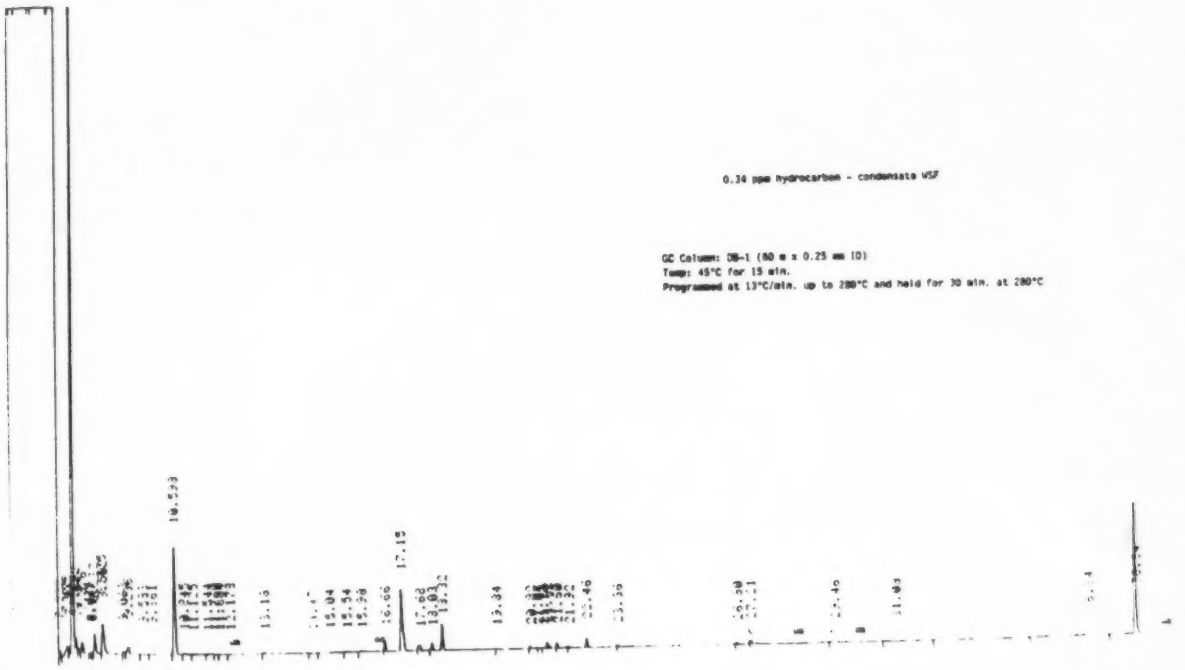
0.34 ppm hydrocarbon - condensate WSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and held for 30 min. at 280°C

Figure C.13. GLC hydrocarbon profile at time 0 from the 96 h 0.55 ppm exposure.

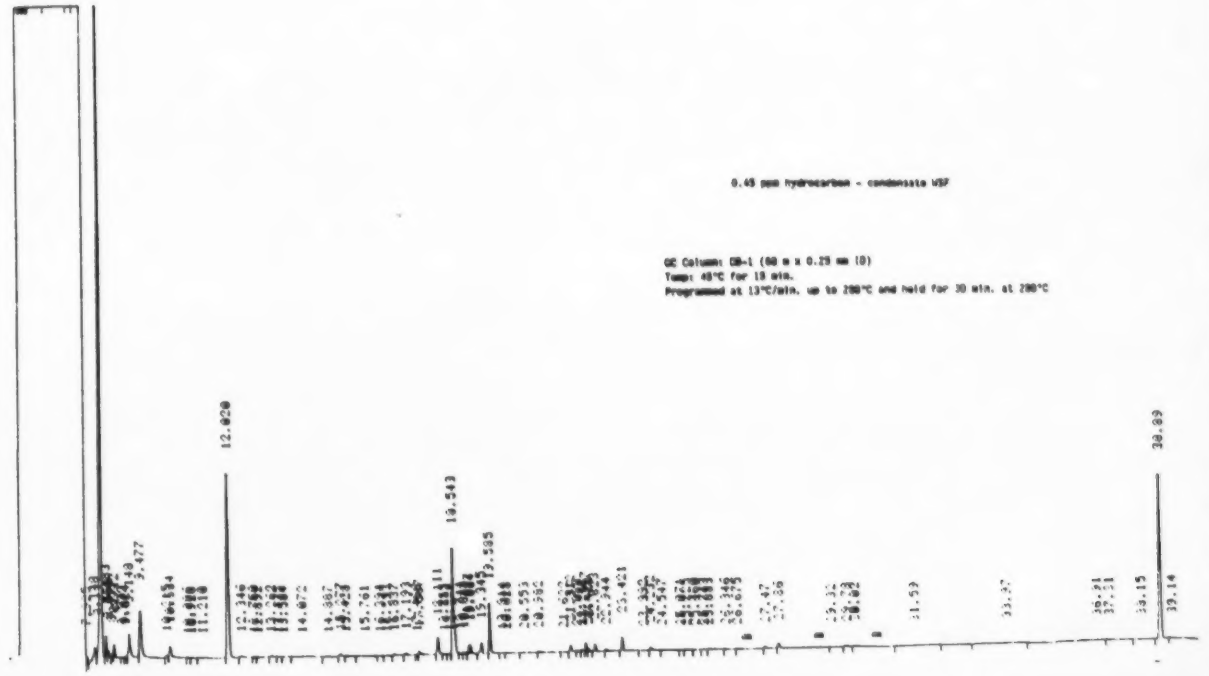
0.45 ppm hydrocarbon - condensate WSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and held for 30 min. at 280°C

Figure C.14. GLC hydrocarbon profile at time 47 h from the 96 h 0.55 ppm exposure.

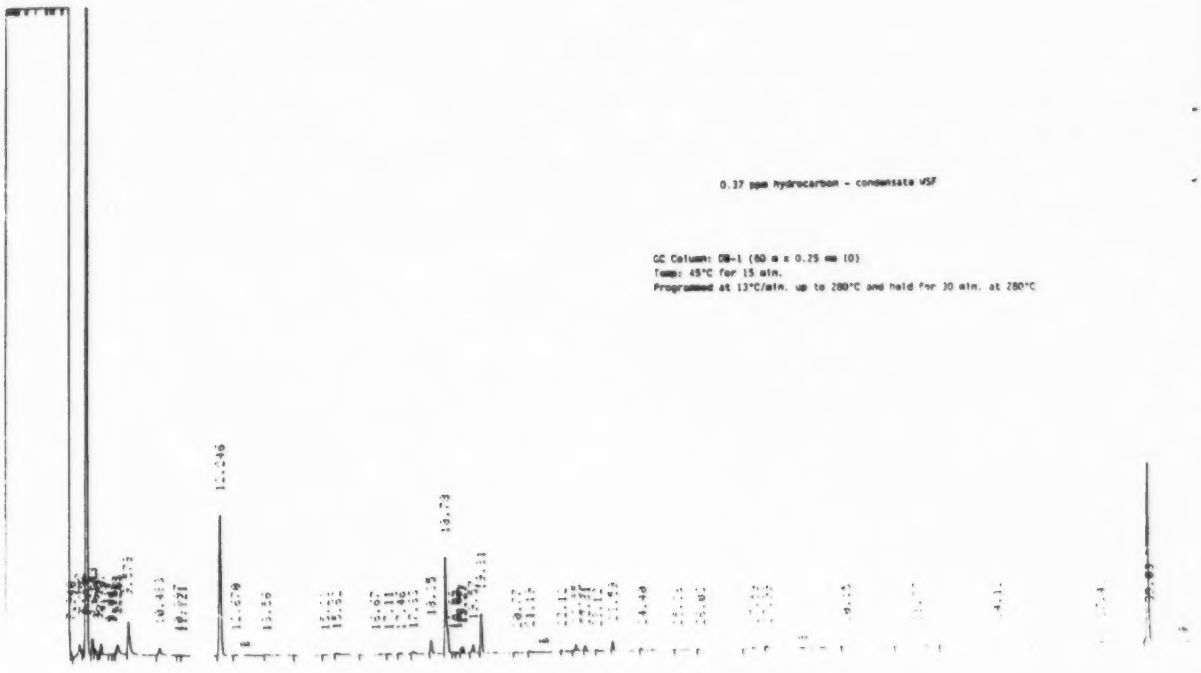
0.37 ppm hydrocarbon - condensate WSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and hold for 30 min. at 280°C

Figure C.15. GLC hydrocarbon profile at time 96 h from the 96 h 0.55 ppm exposure.

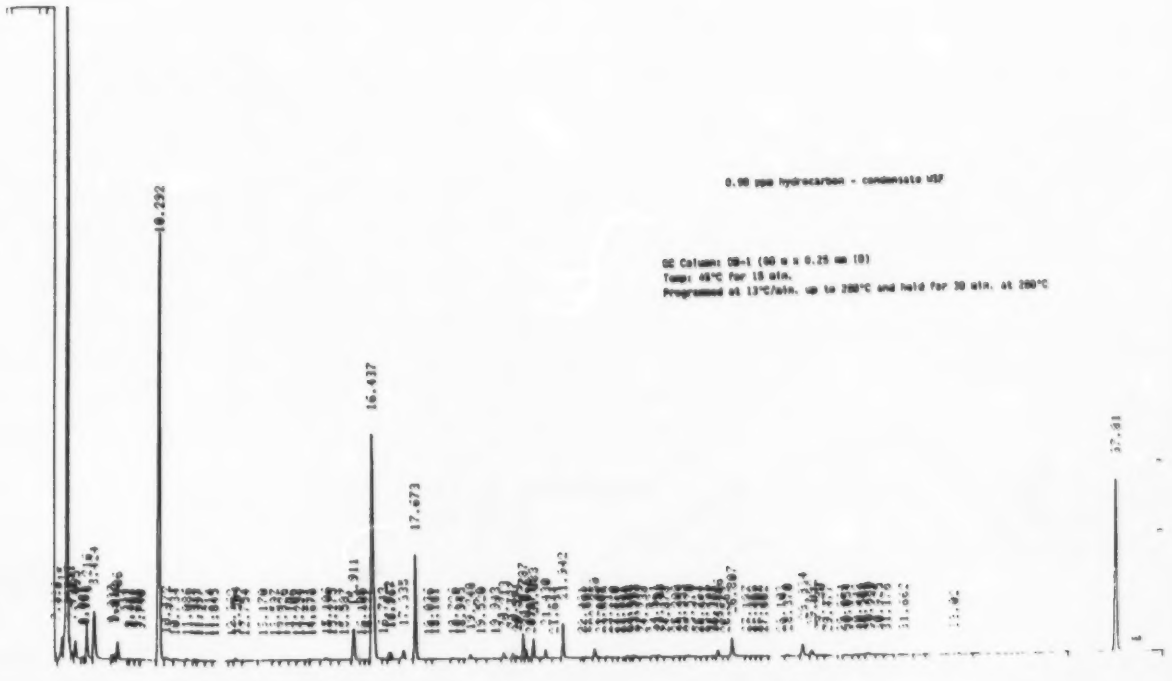
0.98 ppm hydrocarbon - condensate WSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and hold for 30 min. at 280°C
10.292
16.437
17.673
37.161

Figure C.16. GLC hydrocarbon profile at time 0 from the 96 h 0.81 ppm exposure.

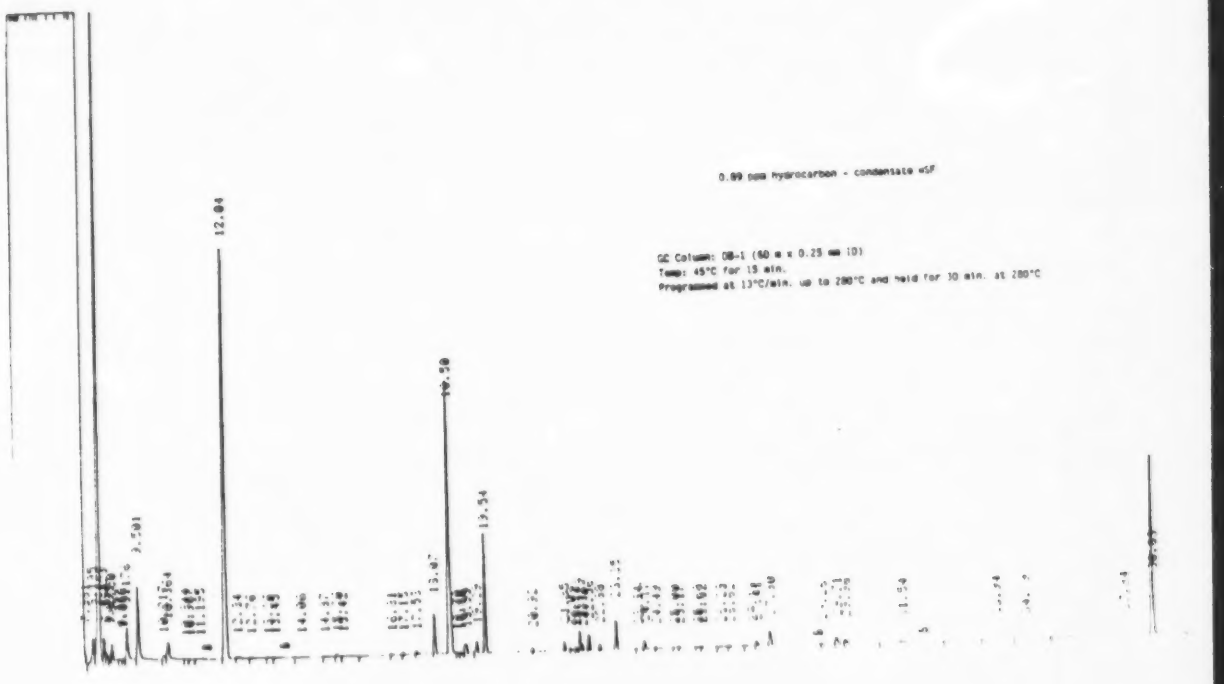

Figure C.17. GLC hydrocarbon profile at time 47 h from the 96 h 0.81 ppm exposure.

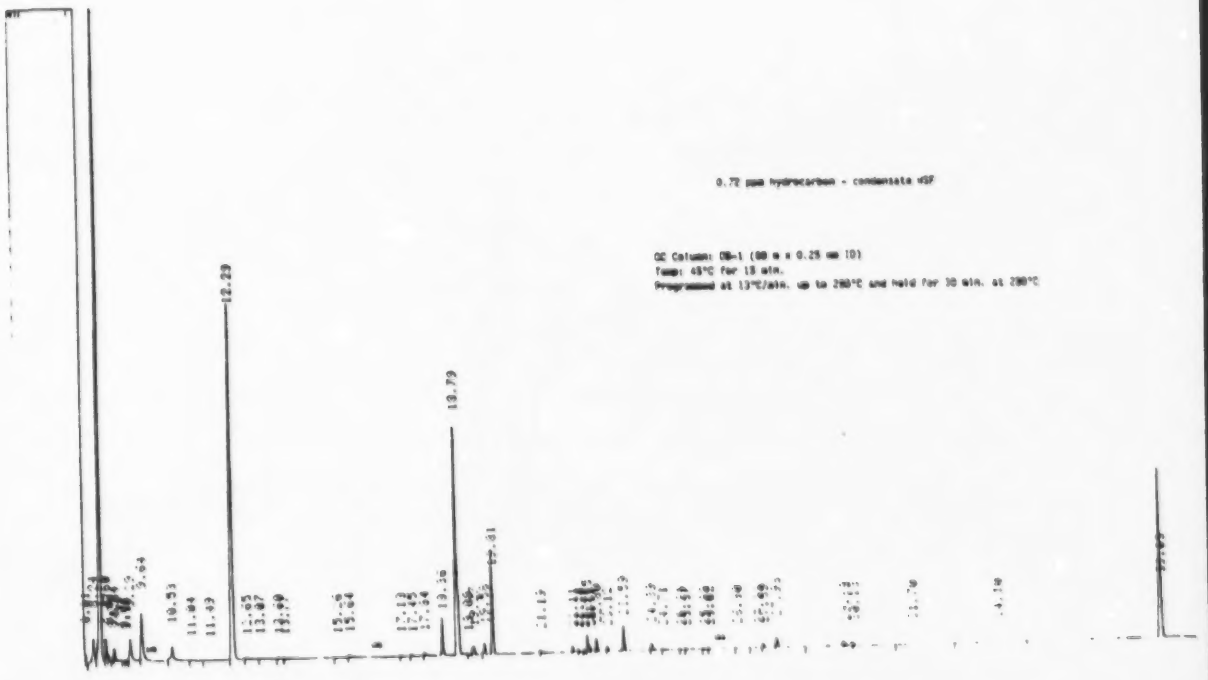

Figure C.18. GLC hydrocarbon profile at time 96 h from the 96 h 0.81 ppm exposure.

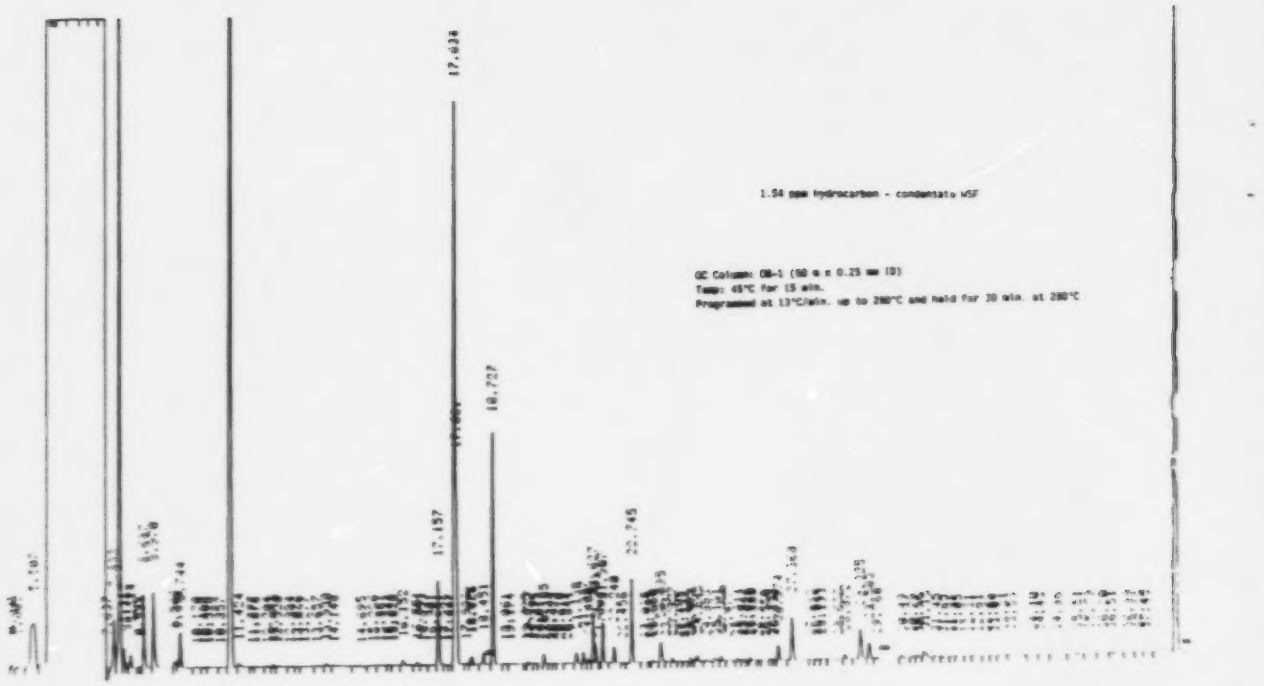

Figure C.19. GLC hydrocarbon profile at time 0 from the 96 h 1.41 ppm exposure.

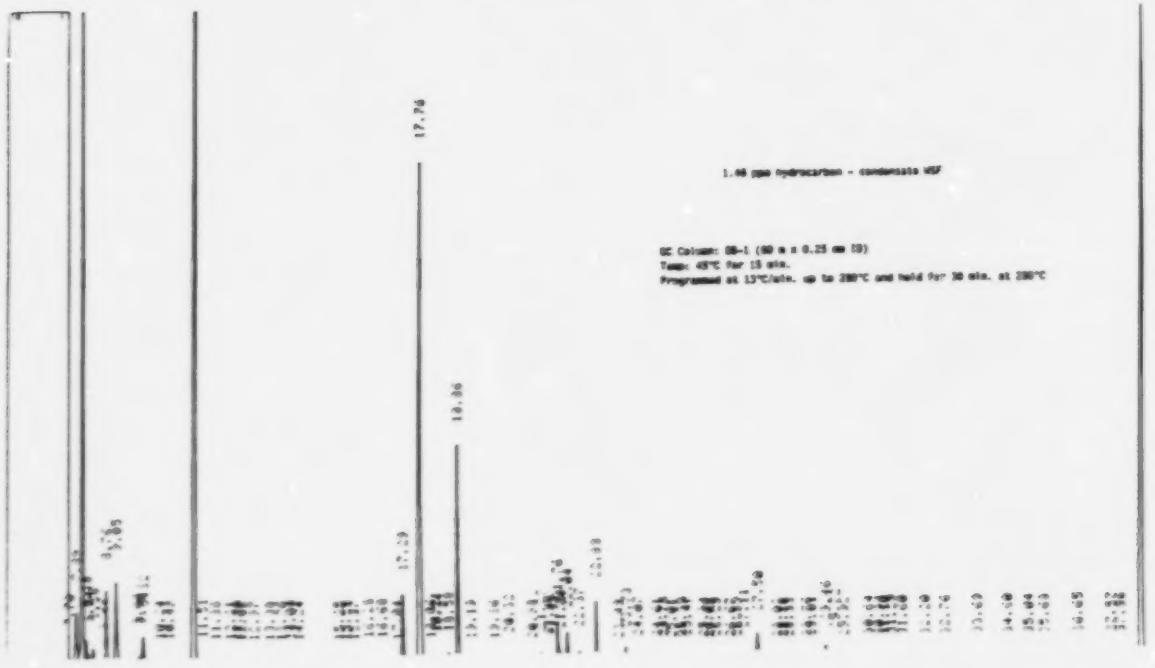

Figure C.20. GLC hydrocarbon profile at time 47 h from the 96 h 1.41 ppm exposure.

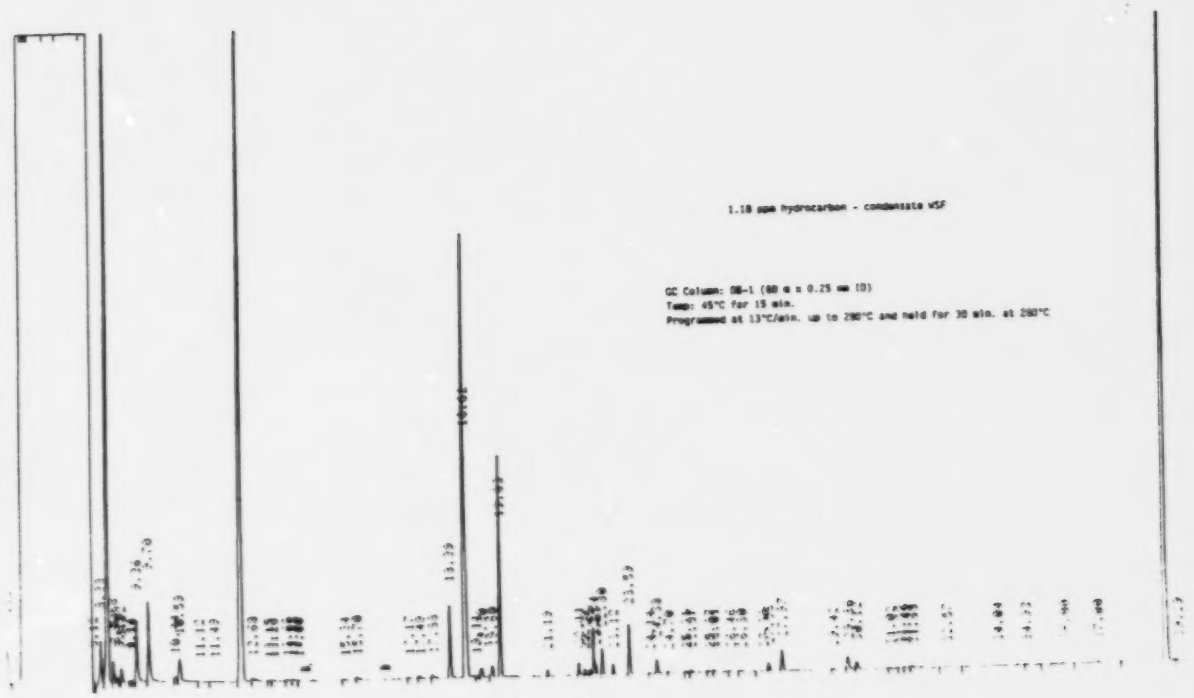
1.18 ppm hydrocarbon - condensate WSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and held for 30 min. at 280°C

Figure C.21. GLC hydrocarbon profile at time 96 h from the 96 h 1.41 ppm exposure.

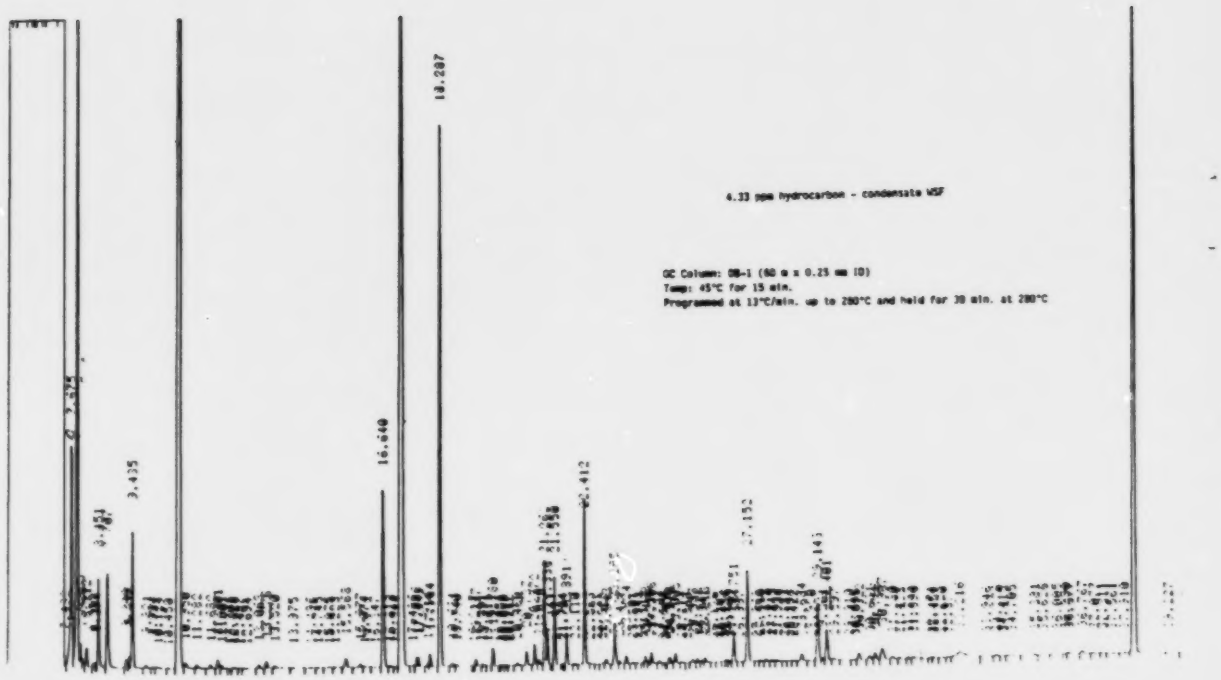
4.33 ppm hydrocarbon - condensate WSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and held for 30 min. at 280°C
3.435
16.640
18.287
26.412

Figure C.22. GLC hydrocarbon profile at time 0 from the 24 h 2.66 ppm exposure (depuration trial).

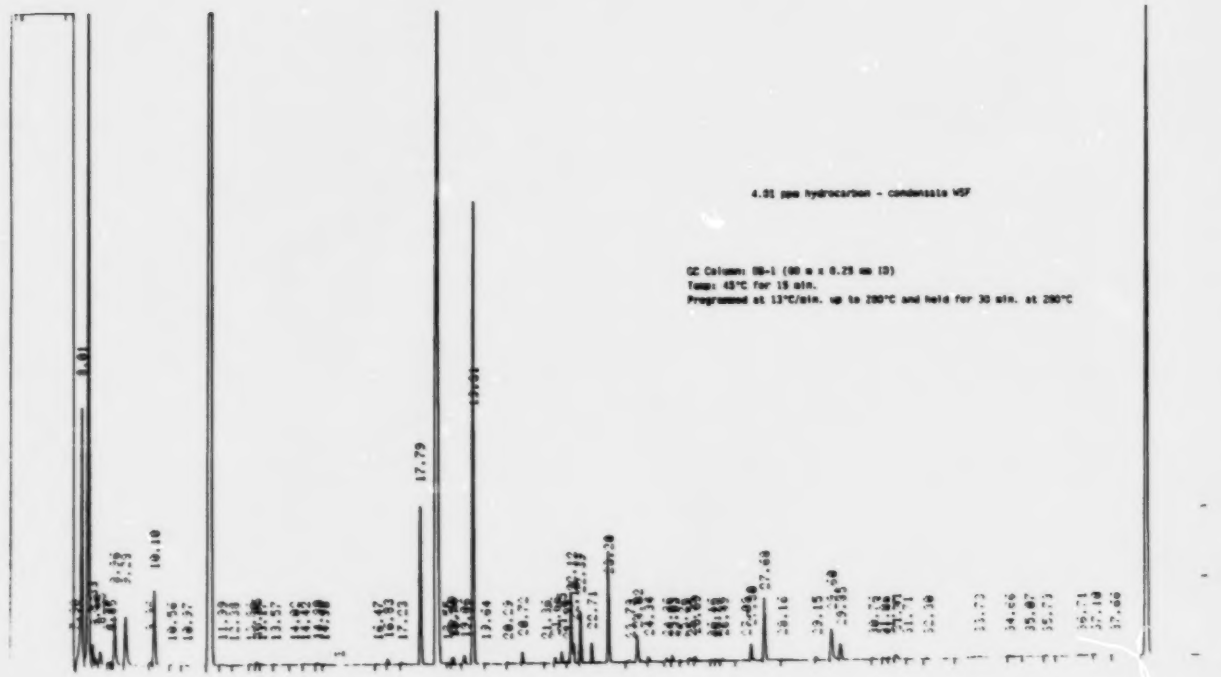
4.01 ppm hydrocarbon - condensate WSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and held for 30 min. at 280°C
17.79

Figure C.23. GLC hydrocarbon profile at time 24 h from the 24 h 2.66 ppm exposure (depuration trial).

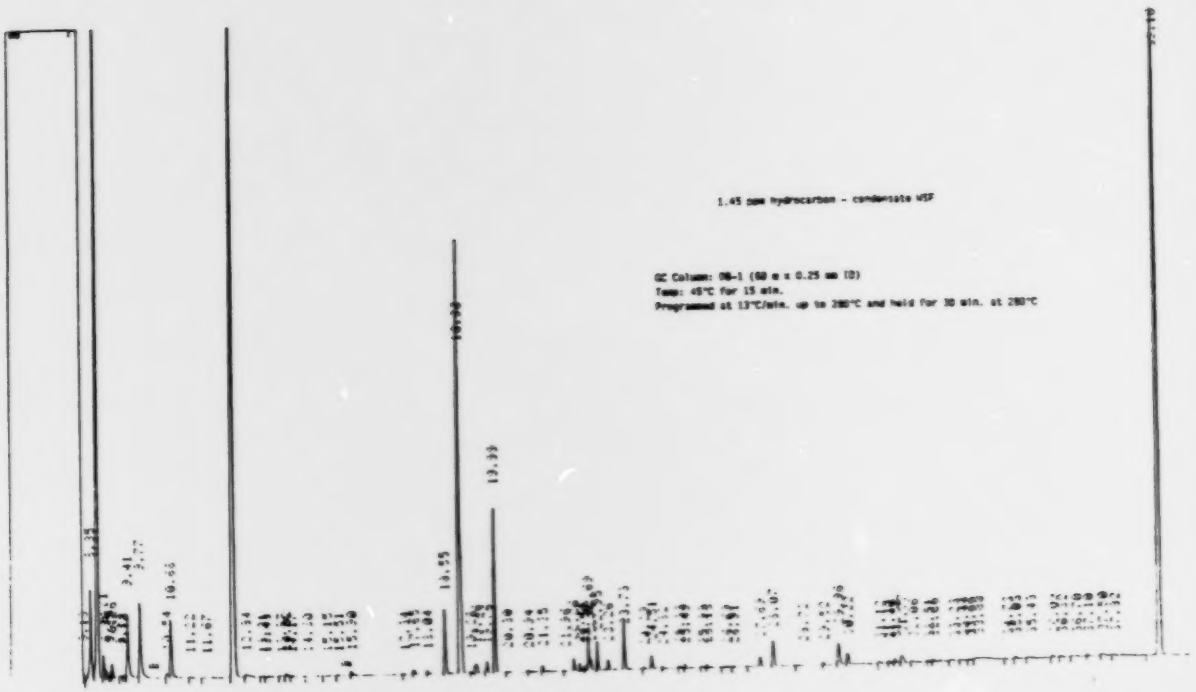
1.45 ppm hydrocarbon - condensate WSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and held for 30 min. at 280°C

Figure C.24. GLC hydrocarbon profile at time 0 from the 96 h 0.92 ppm exposure (depuration trial).

0.90 ppm hydrocarbon - condensate WSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and held for 30 min. at 280°C

Figure C.25. GLC hydrocarbon profile at time 48 h from the 96 h 0.92 ppm exposure (depuration trial).

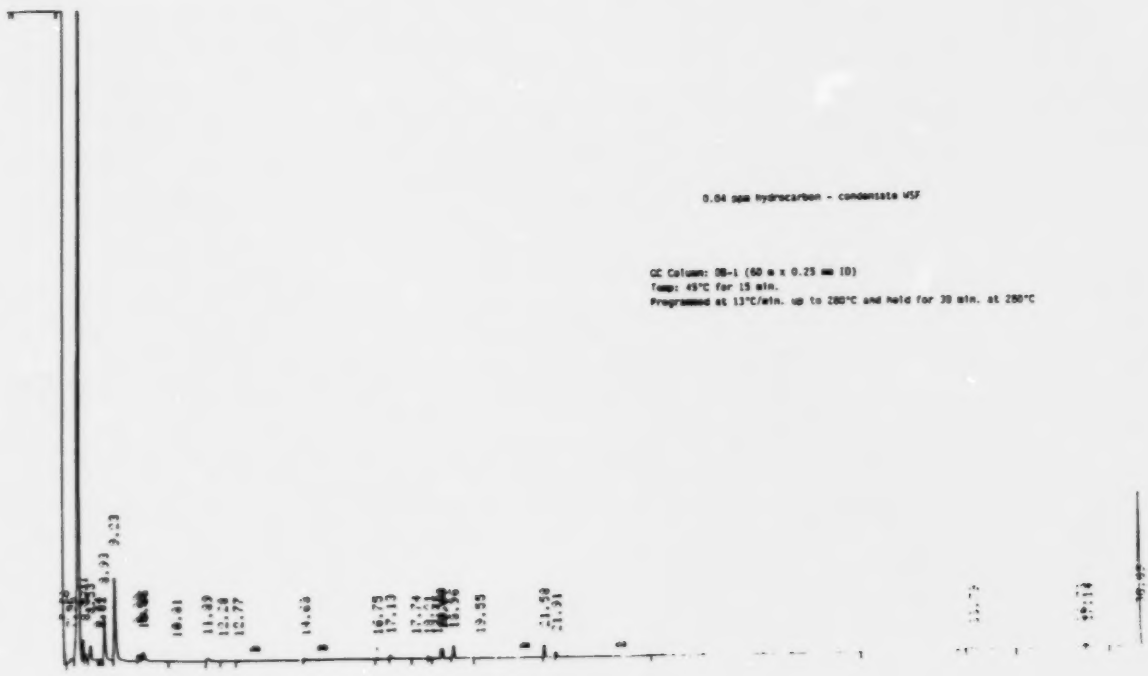
0.04 ppm hydrocarbon - condensate WSF
GC Column: DB-1 (60 m x 0.25 mm ID)
Temp: 45°C for 15 min.
Programmed at 13°C/min. up to 280°C and held for 30 min. at 280°C

Figure C.26. GLC hydrocarbon profile at time 95 h from the 96 h 0.92 ppm exposure (depuration trial).

APPENDIX D

FLUOROMETER CALIBRATION

D.1 General

A Turner Designs Model 10 flow-through fluorometer was used to monitor the hydrocarbon levels in the exposure water. The fluorometer was fitted with a short wavelength oil accessory kit.

Fluorometric analysis is based on the quantitation of the ability of fluorescent materials to absorb light at one wavelength and convert it into light at a longer wavelength. Fluorometric analysis of hydrocarbons accentuates the polynuclear portion of the oil in question. The correlation between fluorescence and GC analysis is expected to be only approximate, especially between different oils and between weathered and unweathered oil (B. Phillips, Turner Designs Ltd., La Jolla, California, pers. comm.).

The sensitivity of the fluorometer is affected by the light which reaches the light detector that is not related to the material being analyzed, and which may vary from sample to sample (Turner Designs Ltd., 1976). The primary sources of such unwanted light are:

- interference from fluorescent materials other than the one being analyzed, and

- interference from light scattered by particulate material in the sample (turbidity).

The response of the fluorometer to varying concentrations of WSF was examined. The first response was a serial dilution of WSF with no scallops present. Another calibration examined the fluorometric

response to varying concentrations of WSF over time and with scallops present in the water.

D.2 Linear Response of the Fluorometer

The response of the fluorometer was checked for linearity by measuring the fluorescence of a serial dilution of a water-soluble fraction (WSF) of condensate. Figure D.1 shows three data sets representing dilutions of the same batch of WSF over a three-day period, i.e., after settling for 48, 72 and 96 h.

The results of this calibration confirmed the linear response of the fluorometer to WSFs as high as 50 ppm ($r = 0.99$).

D.3 Fluorometer Calibration with GC Analysis

Water samples were analyzed for hydrocarbon content by GC throughout each trial. A comparable fluorescence was also recorded. A linear regression was calculated for these pairs and the resulting equation ($y=0.69x + 0.22$) was used to transform all fluorescent readings taken during the trials to express hydrocarbon concentration as ppm (Figure D.2). It is these calibrated fluorescence values which are used to describe the exposure condition for each trial (see Section 3.3).

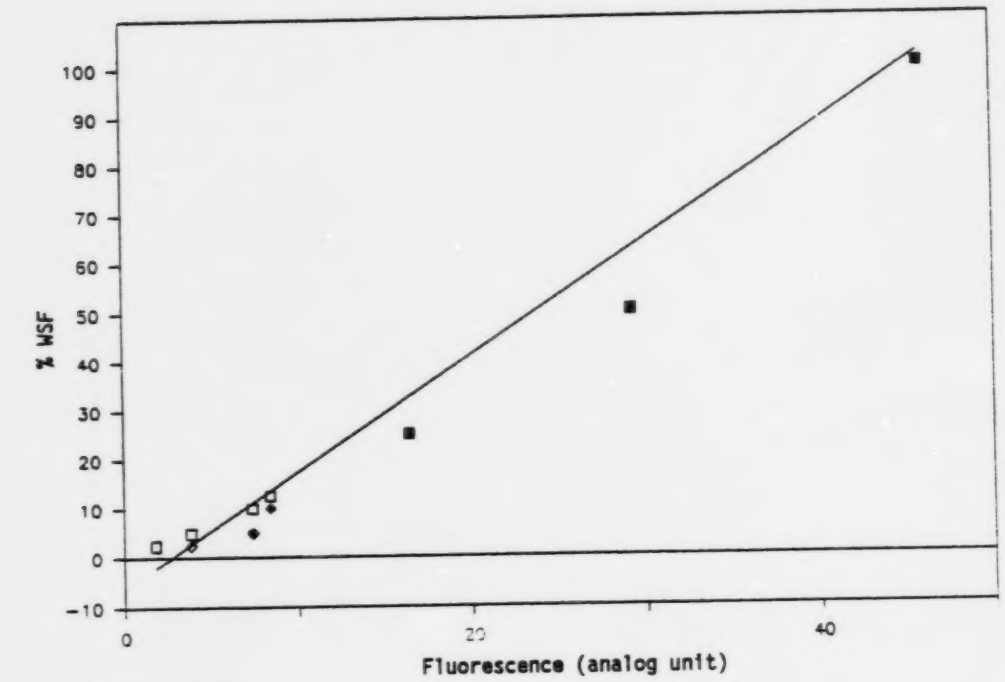

Figure D.1. Linear response of the fluorometer.

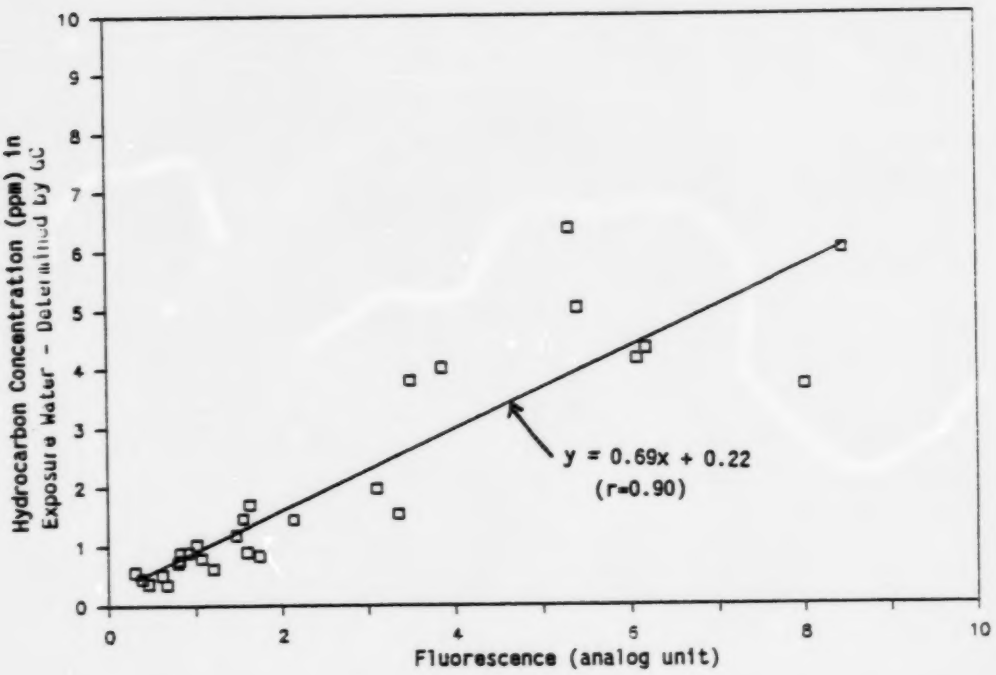

Figure D.2. Relationship between fluorescence values and hydrocarbon concentrations for identical water samples - WSF of condensate.

APPENDIX E

EXPOSURE CONDITIONS

The following tables detail the conditions during each exposure trial. The conditions monitored during each trial were temperature, dissolved oxygen, fluorescence and the flow rate of stock WSF and diluent seawater. A water sample was taken for GC analysis at the beginning and end of each trial and more frequently for selected trials. The GC results were used to calibrate the fluorometer (see Appendix D). The fluorometric data are therefore expressed as anolog units and as ppm hydrocarbon.

Each trial is identified by the exposure time and the hydrocarbon concentration. The hydrocarbon concentration is expressed as a time-weighted average of the calibrated fluorometric data. Each data point is weighted (multiplied) by the length of time it represents, then all are summed and divided by the total exposure time.

Table E.1. Short term exposures.

3 h Exposure at 2.10 ppm Hydrocarbon

Time (h)	Temp. (°C)	D.O. (%)	Fluorescence[1]	Hydrocarbon (ppm)		Flow Rates (mL/min)	
				Calibration[2]	GC	Diluent	WSF
0	3.0	97	3.10	2.36	1.98	980	25.0
1.5	3.0	99	2.75	2.12		980	25.0
3.0	3.0	97	2.30	1.81		990	24.0

3 h Exposure at 3.55 ppm Hydrocarbon

Time (h)	Temp. (°C)	D.O. (%)	Fluorescence[1]	Hydrocarbon (ppm)		Flow Rates (mL/min)	
				Calibration[2]	GC	Diluent	WSF
0	3.0	97	6.09	4.42	4.15	940	51.0
1.5	3.0	97	4.86	3.57		930	51.0
3.0	3.0	95	3.50	2.64	3.80	960	47.0

3 h Exposure at 4.76 ppm Hydrocarbon

Time (h)	Temp. (°C)	D.O. (%)	Fluorescence[1]	Hydrocarbon (ppm)		Flow Rates (mL/min)	
				Calibration[2]	GC	Diluent	WSF
0	3.0	97	8.01	5.75	3.74	930	71
1.5	3.0	97	6.46	4.68		930	70
3.0	3.0	96	5.41	3.95	4.88	950	75

[1]Analog unit from fluorometer scale.
[2]Fluorometer value calibrated with GC data.

Table E.2. Medium term exposures.

24 h Exposure at 0.45 ppm Hydrocarbon

Time (h)	Temp. (°C)	D.O. (%)	Fluorescence[1]	Hydrocarbon (ppm)		Flow Rates (mL/min)	
				Calibration[2]	GC	Diluent	WSF
0	2.0	133	0.62	0.65	0.51	996	6.7
1.5	2.0	88	0.51	0.57		980	5.6
2.5	2.0	109	0.54	0.59		990	5.6
5.0	2.0	120	0.45	0.53		1000	6.7
9.5	2.5	33	0.19	0.35		850	7.0
19.5	2.5	140+	0.28	0.41		1010	5.6
22.0	2.5	138	0.29	0.42		990	5.0
24.0	2.5	125	0.30	0.43	0.56	980	5.7

24 h Exposure at .78 ppm Hydrocarbon

Time (h)	Temp. (°C)	D.O. (%)	Fluorescence[1]	Hydrocarbon (ppm)		Flow Rates (mL/min)	
				Calibration[2]	GC	Diluent	WSF
0	2.0	131	1.63	1.35	1.70	970	15
1.5	2.0	94	1.41	1.19		1000	14.8
2.5	2.25	97	1.27	1.10		970	13.8
5.0	2.25	112	0.92	0.86		990	15
9.5	2.5	39	0.82	0.79		1000	16
19.5	3.0	140+	0.54	0.59		1000	19[3]
22.0	3.0	135	0.78	0.76		1000	15
24.0	2.5	116	0.81	0.78	0.76	990	15.2

24 h Exposure at 3.79 ppm Hydrocarbon

Time (h)	Temp. (°C)	D.O. (%)	Fluorescence[1]	Hydrocarbon (ppm)		Flow Rates (mL/min)	
				Calibration[2]	GC	Diluent	WSF
0	2.5	138	8.46	6.06	6.05	870	82
1.5	2.5	93	6.50	4.71		870	80
2.5	2.5	98	5.76	4.19		910	80
5.0	2.75	103	4.80	3.53		904	82
9.5	3.0	42				1000	80
19.5	3.0	140+	4.94	3.63		900	82
22.0	3.0	135	5.18	3.79		910	80
24.0	3.0	115	5.32	3.89	6.51	920	80

[1]Analog unit from fluorometer scale.
[2]Fluorometer value calibrated with GC data.
[3]WSF was found disconnected; flow was resumed at 19 mL/min for 1.5 h and then reset to 15 mL/min for the duration of the exposure.

Table E.2. (Continued)

24 h Control Exposure (no hydrocarbon)

(Threshold)

Time (h)	Temp. (°C)	D.O. (%)	Fluorescence[1]	Hydrocarbon (ppm)		Flow Rates (mL/min)	
				Calibration[2]	GC	Diluent	WSF
0						1000	0
1.5	2.0	95	0.03				
2.5	2.0	110	0.02				
5.0	2.0	124					
19.5	2.5	140	0.02				
24.0	2.5	110				1000	0

[1]Analog unit from fluorometer scale.
[2]Fluorometer value calibrated with GC data.

Table E.3. Long term exposures.

96 h Exposure at 0.55 ppm Hydrocarbon

Time (h)	Temp. (°C)	D.O. (%)	Fluorescence[1]	Hydrocarbon (ppm)		Flow Rates (mL/min)	
				Calibration[2]	GC	Diluent	WSF
0	2.5	101	0.67	0.68	0.34	994	6.2
2.0	2.5	92	0.57	0.61		1010	5.1
5.5	3.0	98	0.42	0.51		974	6.7
9.0	3.5	99	0.55	0.60		900	8.0
14.0	3.5	95	0.50	0.57		1000	7.0
23.0	3.0	102	0.29	0.42		980	4.4
26.0	3.0	98	0.38	0.48		1000	7.7
29.5	3.0	96	0.49	0.56		1000	6.7
33.0	3.5	95	0.47	0.54		1000	7.8
38.0	3.5	87	0.51	0.57		1000	7.1
47.0	3.25	104	0.38	0.48	0.45	1000	6.3
49.5	3.0	101	0.52	0.58		980	6.4
53.5	3.5	96	0.56	0.61		1000	7.6
56.5	3.5	91	0.54	0.59		1000	7.7
61.5	3.0	91	0.52	0.58		1000	8.0
71.0	3.0	79	0.29	0.42		1040	6.5
74.0	3.5	93	0.47	0.54		1000	8.0
77.5	3.5	88	0.42	0.51		1000	7.4
80.0	3.5	82	0.46	0.54		1000	8.0
86.0	3.0	93	0.55	0.60		1000	7.1
96.0			0.45	0.53	0.37	1000	6.3

[1]Analog unit from fluorometer scale.
[2]Fluorometer value calibrated with GC data.

Table E.3. (Continued)

96 h Exposure at 0.81 ppm Hydrocarbon

Time (h)	Temp. (°C)	D.O. (%)	Fluorescence[1]	Hydrocarbon (ppm)		Flow Rates (mL/min)	
				Calibration[2]	GC	Diluent	WSF
0	2.75	98	1.59	1.32	0.90	960	12.0
2.0	2.5	87	1.34	1.15		1000	14.0
5.5	3.0	96	0.98	0.90		970	13.0
9.0	3.0	99	1.10	0.98		1000	14.0
14.0	3.5	90	1.00	0.91		960	13.0
23.0	3.0	105	0.55	0.60		990	11.5
26.0	3.25	96	0.76	0.74		980	16.0
29.5	3.0	97	0.85	0.81		1000	11.0
33.0	3.5	92	0.89	0.83		970	14.5
38.0	3.5	96	0.89	0.83		970	16.8
47.0	3.25	100	0.82	0.79	0.89	990	14.0
49.5	3.25	102	0.89	0.83		990	15.0
53.5	3.5	100	0.86	0.81		960	14.5
56.5	3.5	92	0.86	0.81		990	15.2
61.5	3.5	91	0.87	0.82		1000	15.0
71.0	3.0	88	0.59	0.63		980	13.0
74.0	3.5	92	0.84	0.80		990	16.0
77.5	3.5	86	0.76	0.74		990	15.5
80.0	3.5	86	0.73	0.72		980	16.0
86.0	3.0	95	0.85	0.81		1000	16.2
96.0			0.80	0.77	0.72	1000	16.0

[1]Analog unit from fluorometer scale.
[2]Fluorometer value calibrated with GC data.

Table E.3. (Continued)

96 h Exposure at 1.41 ppm Hydrocarbon

Time (h)	Temp. (°C)	D.O. (%)	Fluorescence[1]	Hydrocarbon (ppm)		Flow Rates (mL/min)	
				Calibration[2]	GC	Diluent	WSF
0	2.5	101	3.35	2.53	1.54	960	27.0
2.0	2.5	90	2.75	2.12		960	26.5
5.5	3.0	98	1.97	1.58		970	25.0
9.0	3.0	95	2.10	1.67		1000	26.0
14.0	3.0	95	2.00	1.60		970	27.0
23.0	3.0	105	1.28	1.10		970	20.0
26.0	3.0	95	1.65	1.36		970	27.5
29.5	3.0	97	1.74	1.42		980	27.5
33.0	3.5	96	1.81	1.47		960	28.0
38.0	3.5	93	1.84	1.49		980	26.5
47.0	3.25	102	1.55	1.29	1.46	980	26.0
49.5	3.25	100	1.81	1.47		980	26.0
53.5	3.5	101	1.74	1.42		1000	24.0
56.5	3.5	94	1.75	1.43		970	27.0
61.5	3.5	91	1.66	1.37		1000	30.0
71.0	3.0	91	1.31	1.12		980	23.5
74.0	3.5	91	1.65	1.36		980	27.5
77.5	3.5	86	1.62	1.34		990	26.0
80.0	3.5	82	1.54	1.28		1000	28.0
86.0	3.0	95	1.62	1.34		970	28.0
96.0			1.47	1.23	1.18	970	24.0

[1]Analog unit from fluorometer scale.
[2]Fluorometer value calibrated with GC data.

Table E.3. (Continued)

96 h Control Exposure (no hydrocarbon)

Time (h)	Temp. (°C)	D.O. (%)	Fluorescence[1]	Hydrocarbon (ppm)		Flow Rates (mL/min)	
				Calibration[2]	GC	Diluent	WSF
0	2.25	104	0.05			1000	0
5.5	2.5	99					
23.0	3.0	112	0.03			1200	0
26.0	3.0	102					
29.5	3.0	97					
33.0	3.5	97	0.04			1050	0
38.0	3.5	96	0.01			990	0
47.0	3.0	108	0			1060	0
53.5	3.5	104	0.03			990	0
56.5	3.5	98	0			1010	0
71.0	3.0	95	0			1100	0
77.5	3.0	89					
80.0	3.0	96					
86.0	3.0	96	0.01			980	0

[1]Analog unit from fluorometer scale.
[2]Fluorometer value calibrated with GC data.

Table E.4. Depuration trials.

24 h Exposure at 2.72 ppm Hydrocarbon

Time (h)	Temp. (°C)	D.O. (%)	Fluorescence[1]	Hydrocarbon (ppm)		Flow Rates (mL/min)	
				Calibration[2]	GC	Diluent	WSF
0	4.0		5.96	4.33	4.33	950	52
1.5	3.5		4.25	3.15		950	50
4.0	3.5	109	3.00	2.29		930	50
8.0	4.5	86	3.30	2.50		950	56
12.5	5.0	91	3.28	2.48		950	57
20.0	4.5	72	3.93	2.93		960	60
22.0	4.5	76	3.94	2.94		960	57
24.0	4.5	83	3.90	2.91	4.01	950	59

24 h Exposure at 2.68 ppm Hydrocarbon

Time (h)	Temp. (°C)	D.O. (%)	Fluorescence[1]	Hydrocarbon (ppm)		Flow Rates (mL/min)	
				Calibration[2]	GC	Diluent	WSF
0	4.0		6.30	4.57	4.33	960	50
1.5	3.5		4.33	3.21		940	50
4.0	4.0	103	2.84	2.18		950	48
8.0	4.5	89	3.09	2.35		950	56
12.5	5.0	91	3.36	2.54		950	59
20.0	4.5	70	3.78	2.83		950	58
22.0	4.5	78	3.94	2.94		960	60
24.0	4.5	80	3.96	2.95	4.01	950	56

24 h Exposure at 2.57 ppm Hydrocarbon

Time (h)	Temp. (°C)	D.O. (%)	Fluorescence[1]	Hydrocarbon (ppm)		Flow Rates (mL/min)	
				Calibration[2]	GC	Diluent	WSF
0	4.0		6.30	4.57	4.33	940	50
1.5	3.5		4.60	3.39		860	48
4.0	4.0	102	2.95	2.26		940	50
8.0	4.5	90	3.05	2.33		950	55
12.5	5.0	89	3.15	2.39		950	57
20.0	4.5	68	3.40	2.57		950	57
22.0	4.5	76	3.17	2.41		960	56
24.0	4.5	83	3.72	2.79	4.01	950	59

[1]Analog unit from fluorometer scale.
[2]Fluorometer value calibrated with GC data.

Table E.4. (Continued)

24 h Control Exposure (no hydrocarbon)

Time (h)	Temp. (°C)	D.O. (%)	Fluorescence[1]	Hydrocarbon (ppm)		Flow Rates (mL/min)	
				Calibration[2]	GC	Diluent	WSF
0	3.0					1000	0
1.5	3.0		.01				
4.0	3.5	93	.03				
20.0	4.0	97	0				
24.0	4.0	90	0			1000	0

[1]Analog unit from fluorometer scale.
[2]Fluorometer value calibrated with GC data.

Table E.4. (Continued)

96 h Exposure at 0.98 ppm Hydrocarbon

Time (h)	Temp. (°C)	D.O. (%)	Fluorescence[1]	Hydrocarbon (ppm)		Flow Rates (mL/min)	
				Calibration[2]	GC	Diluent	WSF
0	3.0	97	2.21	1.75	1.45	980	18.0
1.0	3.0	100	2.27	1.79			
2.0	3.0	98	1.92	1.55		980	17.0
5.0	3.0	89	1.37	1.17		970	16.0
9.0	3.0	89	1.37	1.17		980	15.0
13.5	3.0	89	1.09	0.97		980	15.2
22.5	3.75	87	0.88	0.83		980	15.5
26.0	3.75	94	0.96	0.88		990	16.0
29.5	4.0	99	1.00	0.91		980	16.0
32.0	3.5	86	1.01	0.92		980	15.5
37.5	3.75	82	1.14	1.01		1000	16.0
46.5	3.75	88	0.97	0.89		960	17.0
48.0			0.88	0.83	0.90		
50.0	4.0	99	0.98	0.90		960	16.0
53.5	4.0	107	1.01	0.92		970	17.0
57.0	4.0	99	1.14	1.01		1000	16.2
62.0	4.0	86	1.08	0.97		980	15.0
70.5	3.0	94	0.97	0.89		1000	17.0
74.0	3.0	109	1.06	0.95		990	17.0
77.5	3.0	100	1.07	0.96		980	17.0
80.0	3.0	73	0.96	0.88			
85.5			1.55	1.29		1100	19.0
95.0	3.0	101	0.29	0.42	0.04	1000	0.0

[1]Analog unit from fluorometer scale.
[2]Fluorometer value calibrated with GC data.

Table E.4. (Continued)

96 h Exposure at 0.89 ppm Hydrocarbon

Time (h)	Temp. (°C)	D.O. (%)	Fluorescence[1]	Hydrocarbon (ppm)		Flow Rates (mL/min)	
				Calibration[2]	GC	Diluent	WSF
0	3.0	97	2.05	1.64	1.45	980	18.0
1.0	3.0	102	1.96	1.57			
2.0	3.0	98	1.71	1.40		960	17.0
5.0	3.0	91	1.22	1.06		970	16.0
9.0	3.0	87	1.13	1.00		950	14.2
13.5	3.0	87	0.83	0.79		980	16.6
22.5	4.0	87	0.86	0.81		960	16.0
26.0	4.0	93	0.98	0.90		1000	18.0
29.5	4.0	97	1.05	0.95		980	17.0
32.0	4.0	82	1.01	0.92		990	16.0
37.5	3.75	87	0.99	0.90		980	16.0
46.5	3.75	88	0.86	0.81		984	15.5
48.0			0.95	0.88	0.90		
50.0	4.0	98	0.82	0.79		980	17.0
53.5	4.0	105	0.95	0.88		990	17.0
57.0	4.0	101	0.97	0.89		990	16.5
62.0	4.0	88	0.99	0.90		1000	17.2
70.5	3.0	91	0.81	0.78		990	14.5
74.0	3.0	107	0.87	0.82		990	17.0
77.5	3.0	102	1.00	0.91		970	20.0
80.0	3.0	78	0.89	0.83			
85.5			1.28	1.10		990	17.0
95.0			0.29	0.42	0.04	990	0

[1]Analog unit from fluorometer scale.
[2]Fluorometer value calibrated with GC data.

Table E.4. (Continued)

96 h Exposure at 0.90 ppm Hydrocarbon

Time (h)	Temp. (°C)	D.O. (%)	Fluorescence[1]	Hydrocarbon (ppm)		Flow Rates (mL/min)	
				Calibration[2]	GC	Diluent	WSF
0	3.0	97	2.17	1.72	1.45	1000	17.0
1.0	3.0	101	2.18	1.72			
2.0	3.0	97	1.87	1.51		980	17.0
5.0	3.0	89	1.33	1.14		980	16.0
9.0	3.0	89	1.13	1.00		950	14.4
13.5	3.0	93	0.83	0.79		970	16.0
22.5	4.0	90	0.77	0.75		1000	13.5
26.0	4.0	92	0.86	0.81		980	15.0
29.5	4.0	96	1.03	0.93		960	19.0
32.0	4.0	83	1.08	0.97		1000	20.0
37.5	4.0	87	1.12	0.99		980	19.0
46.5	3.75	90	0.84	0.80		990	15.0
48.0			0.92	0.86	0.90		
50.0	4.0	97	0.81	0.78		1000	17.0
53.5	4.0	107	0.94	0.87		990	16.5
57.0	4.0	103	0.96	0.88		990	17.2
62.0	4.0	87	0.93	0.86		990	16.4
70.5	3.0	91	0.72	0.72		980	11.0
74.0	3.0	109	0.84	0.80		990	17.0
77.5	3.0	103	0.95	0.88		1000	19.0
80.0	3.0	82	1.29	1.11			
85.5			1.27	1.10		1000	20.0
95.0	3.0	95	0.20	0.36	0.04	1000	0

[1]Analog unit from fluorometer scale.
[2]Fluorometer value calibrated with GC data.

Table E.4. (Continued)

96 h Control Exposure (no hydrocarbon)

Time (h)	Temp. (°C)	D.O. (%)	Fluorescence[1]	Hydrocarbon (ppm)		Flow Rates (mL/min)	
				Calibration[2]	GC	Diluent	WSF
0	3.0	93	0			1000	
2.0	3.0	98					
5.0	3.0	95					
9.0	3.0	93	0.17			1000	
13.5	3.0	97	0.03			1000	
22.5	3.75	94	0			970	
26.0	3.75	95					
29.5	3.75	98	0.06				
32.0	3.75	91	0.02				
37.5	3.75	86					
46.5	3.5	95	0			1000	
53.5	3.5	104	0.01				
57.0	4.0	105	0.01			1000	
62.0	4.0	91	0.01			980	
70.5	3.0	96	0.07			1050	
74.5	3.0	114	0.04			1000	
80.0		85					
95.0			0			1000	

[1]Analog unit from fluorometer scale.
[2]Fluorometer value calibrated with GC data.

APPENDIX F

CALIBRATION OF HYDROCARBON ANALYSIS MADE DURING THE TOXICITY TESTING

F.1 Introduction

The analytical values determined for the toxicity experiments initially were not directly comparable to the values used for the tainting experiments because different analytical techniques were used. The values presented in Section 3.2 are these initial toxicity values calibrated with the fluorometer used during the tainting trials and with the GC.

F.2 Results

Throughout the tainting trials, samples of exposure water were analyzed by GC for total hydrocarbon content and a comparable fluorescence value was recorded using the Turner Fluorometer. All fluorescence values reported for the tainting trials are expressed as ppm hydrocarbon using the equation $y = 0.69x + 0.22$ ($r=0.90$); ($n=28$) as determined in the calibration presented in Appendix D.

On six separate occasions, water samples from the toxicity trials were analyzed both by the Turner Fluorometer and by the freon extraction method described in Section 2.4. The results are summarized below.

Freon Extraction and Fluorescence (ppm hydrocarbon)	Turner Fluorescence (analog unit)
0.236	15.74
6.20	47.75
0.692	23.67
2.31	14.71
3.56	27.60
0.31	11.69

There is a positive linear relationship between these two sets of analytical results described by the equation $y = 5.04x + 12.35$ ($r=0.89$); ($n=6$).

In order to be able to relate the results of the toxicity trials and those from the tainting trials, the exposure conditions during all toxicity trials have been expressed as ppm total hydrocarbon as calibrated with the GC. The results from the "freon method" used during the toxicity trials were transformed to fluorescence units using the freon extraction/fluorometer calibration equation and then transformed to ppm using the fluorometer/GC calibration equation. Therefore, for the toxicity trials, the concentration of the batch (100%) WSFs used ranged from 15 to 22 ppm total hydrocarbon.

Due to concern over the discrepancy between the GC and freon extraction results, the freon method was examined more closely. WSF was subsequently analyzed with freon extraction and an excitation wavelength of 300 nm and emission wavelength of 330 nm (i.e., with the same method used throughout the trials), and with hexane extraction and an excitation wavelength of 270 nm and emission wavelength of 300 nm.

The hexane extraction method resulted in a hydrocarbon concentration 5.24 times that of the freon method. Based on this correction factor, the initial (100% WSF) batch solutions used for the toxicity trials ranged from 10 to 19 ppm hydrocarbon, comparing well with 15 to 22 ppm total hydrocarbon calculated through the calibrations. The hexane extraction method confirmed the general range of hydrocarbons present in the batch solutions used in the toxicity trials.

F.3 Discussion

The stock WSF in the large mixing vessel was analyzed by GC on several occasions throughout the toxicity and tainting trials. The total

hydrocarbon concentration ranged from 42 to 56 ppm and was dominated by low molecular weight aromatic hydrocarbons (see Section 3.1.3). The calculated initial concentration of the batch WSFs used for the toxicity trials indicated a 64% loss in fluorescing hydrocarbons before the toxicity trials began. Figure F.1 is a flow diagram showing how the WSF was handled, prior to being analyzed at CIFT and Seakem, and where losses of hydrocarbons probably occurred.

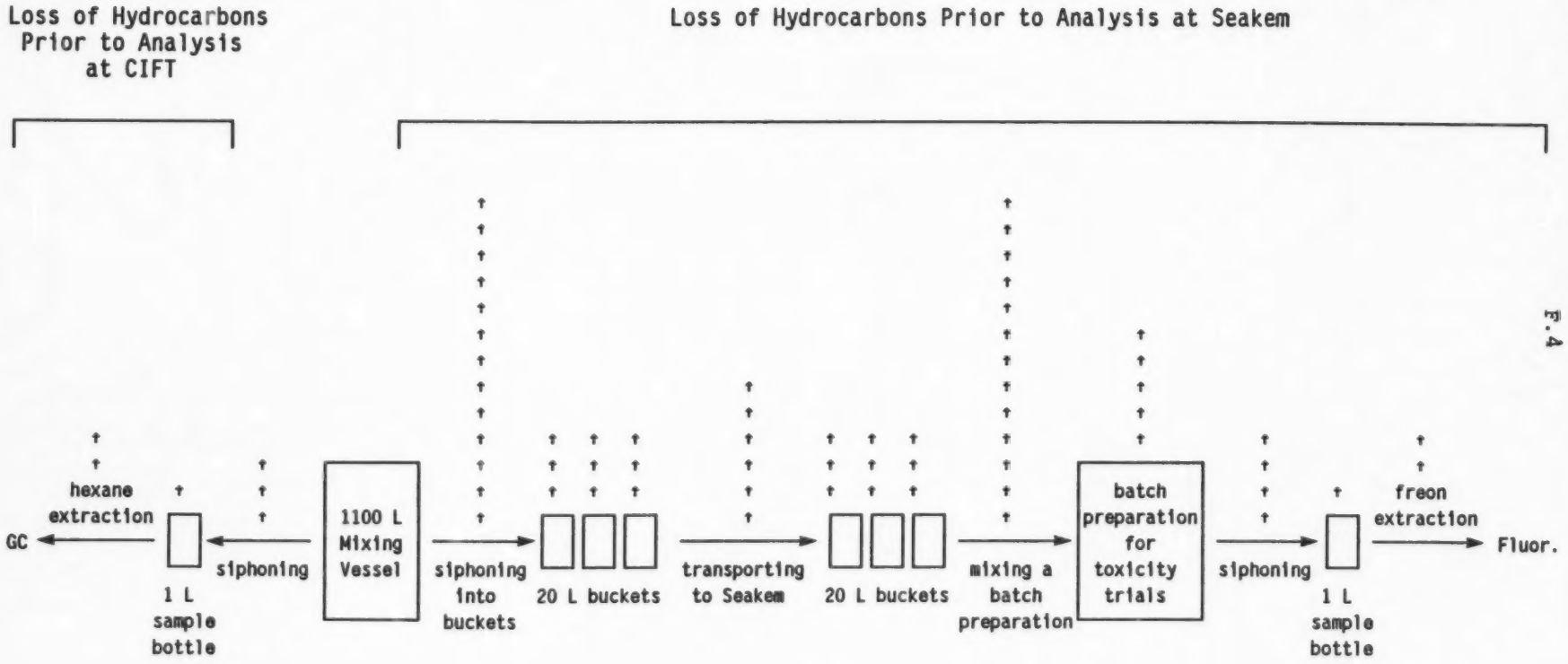

Figure F.1. Potential losses of hydrocarbons from the condensate water-soluble fraction prior to analysis.

APPENDIX G

RECOVERY OF HYDROCARBONS USING THE MURRAY MICROEXTRACTION TECHNIQUE

Recovery studies were conducted with three concentrations (Experiments 1, 2 and 3) of 27 authentic hydrocarbon standards in seawater. The hydrocarbon standards used were representative of those found in the WSFs of the condensate and in general WSFs prepared from crude oils and petroleum products. The amount of spiked hydrocarbon in each of the three experiments is shown in Table G.1. At each level the whole procedure was triplicated and duplicate GC analysis was performed. These results are shown in Tables G.2, G.3 and G.4. As can be seen in these tables, the percentage recovery of this microextraction technique has low variability. The final correction factors applied to all experimental exposure concentrations are shown in Table G.5.

The low molecular weight hydrocarbons such as heptane, isooctane, methylcyclohexane, benzene and toluene needed a higher correction factor because of their lower recovery from the WSF. This low recovery obviously reflected their higher volatility. The higher alkylbenzenes showed a more or less constant recovery factor ranging from 2 to 3.

Table G.1. Amount of spiked hydrocarbon in each of three recovery experiments.

Hydrocarbon Standard	Density	Experiment 1		Experiment 2		Experiment 3	
		Volume (µL)	Weight (µg)	Volume (µL)	Weight (µg)	Volume (µL)	Weight (µg)
Benzene	0.879	2.8	2.46	1.4	1.23	0.8	0.70
Cyclohexane	0.779	7.0	5.45	3.5	2.73	1.8	1.40
2-Methylhexane	0.678	18.0	12.20	9.0	6.10	4.5	3.05
Isooctane	0.692	5.4	3.74	2.8	1.94	1.4	0.97
Heptane	0.684	7.2	4.92	3.6	2.46	1.8	1.23
Methylcyclohexane	0.769	7.0	5.39	3.5	2.70	1.8	1.39
Toluene	0.867	1.5	1.30	0.8	0.69	0.4	0.35
Octane	0.703	3.6	2.53	1.8	1.27	1.0	0.71
Ethylbenzene	0.867	1.0	0.87	0.6	0.52	0.4	0.35
m-Xylene	0.864	2.8	2.42	1.4	1.21	0.8	0.69
p-Xylene	0.861	2.8	2.41	1.4	1.21	0.8	0.69
o-Xylene	0.880	2.8	2.46	1.4	1.23	0.8	0.70
Nonane	0.718	3.6	2.58	1.8	1.29	1.0	0.72
Isopropylbenzene	0.864	1.0	0.86	0.6	0.52	0.4	0.35
Propylbenzene	1.491	1.0	1.49	0.6	0.89	0.4	0.59
1,3,5-Trimethylbenzene	0.865	1.0	0.86	0.6	0.52	0.4	0.35
1,2,4-Trimethylbenzene	0.876	1.0	0.88	0.6	0.53	0.4	0.35
Decane	0.730	1.8	1.31	1.0	0.73	0.6	0.44
1,2,3-Trimethylbenzene	0.894	1.0	0.89	0.6	0.53	0.4	0.35
Undecane	0.740	1.8	1.33	1.0	0.74	0.6	0.44
1,2,4,5-Tetramethylbenzene		9.0	0.98	4.5	0.49	2.2	0.24
1,2,3,5-Tetramethylbenzene	0.889	1.0	0.89	0.6	0.53	0.4	0.35
Naphthalene		8.0	1.00	4.0	0.50	2.0	0.25
Dodecane	0.749	1.8	1.35	1.0	0.75	0.6	0.45
2-Methylnaphthalene		1.0	1.00	0.5	0.50	0.2	0.20
Tridecane	0.756	1.8	1.36	1.0	0.76	0.6	0.46
1-Methylnaphthalene	1.020	0.6	0.61	0.4	0.41	0.2	0.21

Table G.2. Recovery correction factors for amount of hydrocarbons spiked in Experiment #1.

Hydrocarbon	Actual Amount Spiked (µg)	Experimental Recovery (µg)						%Recovery (Mean±S.D.)	Recovery Correction Factor (Mean±S.D.)
		Experiment 1A		Experiment 1B		Experiment 1C			
		Analysis 1	Analysis 2	Analysis 1	Analysis 2	Analysis 1	Analysis 2		
Benzene	2.46	0.25	0.25	0.24	0.24	0.23	0.25	9.88 ± 0.40	10.14 ± 0.42
Cyclohexane	5.45	0.94	0.99	1.07	1.08	0.94	1.04	18.53 ± 1.13	5.41 ± 0.33
2-Methylhexane	12.20	1.62	1.68	2.03	2.03	1.77	1.95	15.13 ± 1.46	6.66 ± 0.65
Isooctane	3.74	0.49	0.51	0.59	0.59	0.55	0.59	14.75 ± 1.16	6.82 ± 0.55
Heptane	4.92	0.72	0.75	0.88	0.88	0.80	0.87	16.10 ± 1.04	6.23 ± 0.40
Methylcyclohexane	5.39	0.97	1.01	1.13	1.14	1.01	1.12	19.61 ± 1.31	6.23 ± 0.40
Toluene	1.30	0.27	0.28	0.28	0.28	0.29	0.31	21.93 ± 1.19	4.57 ± 0.23
Octane	2.53	0.53	0.56	0.73	0.74	0.64	0.69	25.62 ± 3.48	3.97 ± 0.57
Ethylbenzene	0.87	0.30	0.32	0.32	0.33	0.31	0.34	36.37 ± 1.54	2.75 ± 0.12
m-Xylene	2.42	0.88	1.02	1.08	1.10	0.96	0.99	41.58 ± 3.25	2.42 ± 0.20
p-Xylene	2.41	0.78	0.74	0.77	0.79	0.74	0.87	32.38 ± 1.96	3.10 ± 0.18
o-Xylene	2.46	0.82	0.88	0.89	0.91	0.83	0.91	35.50 ± 1.57	2.82 ± 0.13
Nonane	2.58	1.07	1.15	1.34	1.38	1.24	1.37	48.72 ± 4.96	2.07 ± 0.22
Isopropylbenzene	0.86	0.32	0.35	0.36	0.37	0.32	0.35	40.07 ± 2.25	2.50 ± 0.14
Propylbenzene	1.49	0.42	0.45	0.48	0.49	0.44	0.49	31.07 ± 1.87	3.23 ± 0.20
1,3,5-Trimethylbenzene	0.86	0.43	0.46	0.46	0.48	0.44	0.48	53.25 ± 2.44	1.88 ± 0.09
1,2,4-Trimethylbenzene	0.88	0.44	0.47	0.47	0.49	0.46	0.50	53.67 ± 2.48	1.86 ± 0.08
Decane	1.31	0.91	0.98	0.98	1.01	0.91	0.99	73.43 ± 3.28	1.36 ± 0.06
1,2,3-Trimethylbenzene	0.89	0.37	0.40	0.43	0.44	0.40	0.44	46.60 ± 3.12	2.16 ± 0.15
Undecane	1.33	1.12	1.21	1.16	1.19	1.06	1.16	86.61 ± 3.91	1.15 ± 0.06
1,2,4,5-Tetramethylbenzene	0.98	0.47	0.51	0.56	0.57	0.63	0.68	58.04 ± 8.06	1.75 ± 0.24
1,2,3,5-Tetramethylbenzene	0.89	0.51	0.54	0.58	0.57	0.57	0.63	63.38 ± 4.80	1.58 ± 0.12
Naphthalene	1.00	0.28	0.31	0.37	0.38	0.38	0.42	35.70 ± 5.23	2.86 ± 0.45
Dodecane	1.35	1.23	1.31	1.25	1.27	1.17	1.28	92.74 ± 3.52	1.08 ± 0.04
2-Methylnaphthalene	1.00	0.50	0.54	0.54	0.55	0.59	0.65	56.16 ± 5.18	1.79 ± 0.16
Tridecane	1.36	1.29	1.37	1.33	1.35	1.25	1.36	97.21 ± 3.52	1.03 ± 0.04
1-Methylnaphthalene	0.61	0.44	0.47	0.44	0.44	0.42	0.47	73.17 ± 3.10	1.37 ± 0.05

Table G.3. Recovery correction factors for amount of hydrocarbons spiked in Experiment #2.

Hydrocarbon	Actual Amount Spiked (µg)	Experimental Recovery (µg)						%Recovery (Mean±S.D.)	Recovery Correction Factor (Mean±S.D.)
		Experiment 2A		Experiment 2B		Experiment 2C			
		Analysis 1	Analysis 2	Analysis 1	Analysis 2	Analysis 1	Analysis 2		
Benzene	1.23	0.10	0.10	0.11	.10	0.10	0.11	8.29 ± 0.37	12.09 ± 0.55
Cyclohexane	2.73	0.41	0.40	0.47	0.41			15.44 ± 1.18	6.50 ± 0.46
2-Methylhexane	6.10	0.71	0.73	0.77	0.73	0.76	0.79	12.23 ± 0.51	8.19 ± 0.34
Isooctane	1.94	0.20	0.21	0.23	0.21	0.20	0.21	10.81 ± 0.57	9.27 ± 0.46
Heptane	2.46	0.28	0.28	0.31	0.28	0.25	0.27	11.27 ± 0.73	8.90 ± 0.58
Methylcyclohexane	2.70	0.39	0.40	0.46	0.43	0.45	0.47	16.05 ± 1.27	6.26 ± 0.51
Toluene	0.69	0.12	0.13	0.14	0.13	0.15	0.15	19.70 ± 1.88	5.11 ± 0.47
Octane	1.27	0.16	0.16	0.19	0.17	0.19	0.20	13.92 ± 1.47	7.25 ± 0.76
Ethylbenzene	0.52	0.15	0.16	0.20	0.18	0.19	0.20	34.52 ± 3.94	2.93 ± 0.36
m-Xylene	1.21	0.40	0.40		0.45	0.45	0.46	35.69 ± 2.44	2.82 ± 0.20
p-Xylene	1.21	0.39	0.40		0.39	0.45	0.47	34.72 ± 3.18	2.90 ± 0.26
o-Xylene	1.23	0.38	0.39	0.43	0.40	0.45	0.47	34.20 ± 3.06	2.94 ± 0.26
Nonane	1.29	0.28	0.29	0.36	0.33	0.35	0.35	35.40 ± 2.53	3.97 ± 0.42
Isopropylbenzene	0.52	0.18	0.18	0.18	0.17	0.20	0.21	35.82 ± 2.55	2.80 ± 0.20
Propylbenzene	0.89	0.23	0.24	0.27	0.25	0.24	0.24	27.43 ± 1.57	3.66 ± 0.20
1,3,5-Trimethylbenzene	0.52	0.20	0.21	0.28	0.26	0.25	0.26	46.18 ± 5.96	2.20 ± 0.30
1,2,4-Trimethylbenzene	0.53	0.24	0.25	0.27	0.25	0.25	0.26	47.99 ± 1.85	2.08 ± 0.08
Decane	0.73	0.27	0.28	0.37	0.35	0.34	0.36	44.92 ± 5.60	2.26 ± 0.30
1,2,3-Trimethylbenzene	0.53	0.20	0.20	0.25	0.23	0.25	0.26	43.75 ± 5.37	2.32 ± 0.30
Undecane	0.74	0.46	0.48	0.53	0.50	0.53	0.55	68.69 ± 4.59	1.46 ± 0.10
1,2,4,5-Tetramethylbenzene	0.49	0.20	0.21		0.23	0.26	0.26	47.22 ± 5.89	2.14 ± 0.26
1,2,3,5-Tetramethylbenzene	0.53	0.26	0.27		0.25	0.31	0.32	53.22 ± 6.00	1.90 ± 0.20
Naphthalene	0.50	0.15	0.15	0.17	0.16	0.17	0.18	32.00 ± 2.36	3.14 ± 0.23
Dodecane	0.75	0.59	0.61	0.64	0.61	0.64	0.66	83.42 ± 3.45	1.20 ± 0.05
2-Methylnaphthalene	0.50	0.20	0.21		0.22	0.21	0.22	42.30 ± 1.84	2.37 ± 0.10
Tridecane	0.76	0.64	0.67		0.68	0.68	0.70	88.59 ± 3.12	1.13 ± 0.04
1-Methylnaphthalene	0.41	0.19	0.19	0.28	0.27	0.25	0.25	58.66 ± 9.15	1.74 ± 0.29

Table G.4. Recovery correction factors for amount of hydrocarbons spiked in Experiment #3.

Hydrocarbon	Actual Amount Spiked (µg)	Experimental Recovery (µg)								%Recovery (Mean±S.D.)	Recovery Correction Factor (Mean±S.D.)
		Experiment 3A		Experiment 3B		Experiment 3C		Experiment 3D			
		Analysis 1	Analysis 2	Analysis 1	Analysis 2	Analysis 1	Analysis 2	Analysis 1	Analysis 2		
Benzene	0.70	0.06	0.06			0.05	0.05	0.05	0.05	7.91± 0.53	12.69± 0.82
Cyclohexane	1.40							0.14	0.18	11.23± 2.05	9.06± 1.65
2-Methylhexane	3.05	0.32	0.34	0.31	0.31	0.30	0.29	0.29	0.31	10.11± 0.52	9.91± 0.50
Isooctane	0.97	0.09	0.10			0.08	0.07	0.09	0.10	8.96± 1.00	11.28± 1.33
Heptane	*1.23*	*0.09*	*0.10*	*0.08*	*0.08*	*0.10*	*0.11*	*7.63*	*1.15*	*7.63 ± 1.15*	*13.37± 2.08*
Methylcyclohexane	1.39	0.21	0.23			0.20	0.19	0.18	0.19	14.31± 1.19	7.03± 0.56
Toluene	0.35	0.07	0.07			0.06	0.06	0.06	0.06	18.07± 1.41	5.56± 0.42
Octane	0.71	0.07	0.08	0.07	0.07	0.07	0.07	0.06	0.07	9.87± 0.52	10.16± 0.55
Ethylbenzene	0.35	0.14	0.14	0.12	0.12	0.12	0.12	0.11	0.12	35.13± 3.42	2.87± 0.26
m-Xylene	0.69	0.27	0.28	0.28	0.27	0.28	0.24	0.19	0.21	36.52± 4.84	2.79± 0.42
p-Xylene	0.69	0.26	0.28	0.25	0.25		0.22	0.19	0.20	34.09± 4.38	2.98± 0.40
o-Xylene	0.70	0.25	0.27	0.24	0.24	0.23	0.22	0.18	0.20	32.43± 3.93	3.13± 0.40
Nonane	0.72	0.11	0.12	0.11	0.11	0.11	0.10	0.09	0.10	14.72± 1.09	6.83± 0.51
Isopropylbenzene	0.35	0.12	0.12	0.12	0.12	0.12	0.11	0.10	0.10	32.60± 2.96	3.09± 0.31
Propylbenzene	0.59	0.20	0.22	0.17	0.17	0.14	0.13	0.13	0.15	27.72± 5.32	3.72± 0.66
1,3,5-Trimethylbenzene	0.35	0.17	0.18	0.17	0.17	0.20	0.19	0.12	0.13	47.53± 8.08	2.17± 0.42
1,2,4-Trimethylbenzene	0.35	0.21	0.23	0.20	0.20	0.18	0.17	0.13	0.14	52.37± 9.34	1.97± 0.38
Decane	0.44	0.12	0.12	0.12	0.12	0.11	0.10	0.10	0.11	25.74± 2.03	3.90± 0.31
1,2,3-Trimethylbenzene	0.35	0.15	0.16	0.16	0.16	0.1	0.15	0.14	0.15	44.04± 1.82	2.28± 0.10
Undecane	0.44	0.19	0.20	0.23	0.23	0.21	0.20	0.19	0.20	47.04± 3.42	2.14± 0.16
1,2,4,5-Tetramethylbenzene	0.24	0.11	0.11	0.09	0.09	0.11	0.10	0.08	0.08	39.60± 5.39	2.57± 0.35
1,2,3,5-Tetramethylbenzene	0.35	0.20	0.21	0.18	0.18	0.20	0.16	0.14	0.15	50.96± 7.76	2.01± 0.33
Naphthalene	0.25	0.07	0.08	0.07	0.07	0.07	0.06	0.05	0.06	26.96± 3.37	3.76± 0.49
Dodecane	0.45	0.33	0.35	0.34	0.33	0.30	0.29	0.27	0.29	69.17± 6.37	1.46± 0.14
2-Methylnaphthalene	0.20	0.08	0.09	0.08	0.07	0.07	0.07			38.08± 3.05	2.64± 0.20
Tridecane	0.46	0.39	0.41	0.46	0.46	0.38	0.36	0.33	0.35	85.31±10.18	1.19± 0.14
1-Methylnaphthalene	0.21	0.17	0.17	0.14	0.14	0.11	0.11	0.13	0.14	65.49±10.78	1.56± 0.26

Table G.5. Final correction factors for all experimental exposure concentrations.

Hydrocarbon Standard	GLC System Response Correction Factor	Final Correction Factor			
		Experiment 1	Experiment 2	Experiment 3	Average
Benzene	0.91	9.23 ± 0.38	11.00 ± 0.50	11.54 ± 0.75	11.90 ± 1.84
Cyclohexane	1.16	6.28 ± 0.39	7.54 ± 0.54	10.50 ± 1.92	10.64 ± 2.63
2-Methylhexane	1.03	6.86 ± 0.67	8.44 ± 0.36	10.21 ± 0.52	8.50 ± 1.68
Isooctane	0.98	6.68 ± 0.54	9.09 ± 0.46	11.06 ± 1.30	8.94 ± 2.19
Heptane	0.94	5.86 ± 0.38	8.37 ± 0.54	12.57 ± 1.95	8.93 ± 3.39
Methylcyclohexane	1.05	5.37 ± 0.36	6.57 ± 0.54	7.38 ± 0.59	6.44 ± 1.01
Toluene	0.96	4.38 ± 0.22	4.91 ± 0.45	5.34 ± 0.41	4.87 ± 0.48
Octane	1.07	4.24 ± 0.61	7.76 ± 0.82	10.87 ± 0.59	7.62 ± 3.32
Ethylbenzene	0.94	2.59 ± 0.11	2.75 ± 0.33	2.70 ± 0.24	2.68 ± 0.08
m-Xylene	0.91	2.20 ± 0.18	2.56 ± 0.18	2.54 ± 0.38	2.43 ± 0.20
p-Xylene	0.91	2.81 ± 0.16	2.64 ± 0.23	2.70 ± 0.35	2.72 ± 0.09
o-Xylene	0.91	2.57 ± 0.12	2.68 ± 0.23	2.84 ± 0.37	2.70 ± 0.14
Nonane	1.07	2.21 ± 0.23	4.25 ± 0.45	7.30 ± 0.54	4.56 ± 2.56
Isopropylbenzene	0.94	2.35 ± 0.13	2.64 ± 0.18	2.91 ± 0.29	2.63 ± 0.28
Propylbenzene	0.95	3.07 ± 0.19	3.47 ± 0.20	3.53 ± 0.62	3.06 ± 0.46
1,3,5-Trimethylbenzene	0.93	1.75 ± 0.08	2.04 ± 0.28	2.02 ± 0.39	1.94 ± 0.16
1,2,4-Trimethylbenzene	0.91	1.70 ± 0.08	1.90 ± 0.07	1.79 ± 0.35	1.80 ± 0.10
Decane	1.06	1.45 ± 0.07	2.40 ± 0.32	4.14 ± 0.33	2.66 ± 1.36
1,2,3-Trimethylbenzene	0.97	2.09 ± 0.14	2.25 ± 0.29	2.21 ± 0.09	2.18 ± 0.08
Undecane	1.08	1.25 ± 0.06	1.58 ± 0.11	2.31 ± 0.17	1.71 ± 0.54
1,2,4,5-Tetramethylbenzene	0.98	1.72 ± 0.24	2.10 ± 0.26	2.52 ± 0.35	2.11 ± 0.40
1,2,3,5-Tetramethylbenzene	0.98	1.56 ± 0.11	1.86 ± 0.20	1.96 ± 0.32	1.79 ± 0.21
Naphthalene	0.95	2.72 ± 0.43	2.98 ± 0.22	3.57 ± 0.45	3.09 ± 0.40
Dodecane	1.10	1.19 ± 0.04	1.32 ± 0.05	1.60 ± 0.15	1.37 ± 0.21
2-Methylnaphthalene	0.93	1.67 ± 0.14	2.20 ± 0.10	2.45 ± 0.19	2.10 ± 0.40
Tridecane	1.11	1.14 ± 0.04	1.26 ± 0.04	1.32 ± 0.16	1.24 ± 0.09
1-Methylnaphthalene		1.37 ± 0.05	1.74 ± 0.29	1.56 ± 0.26	1.55 ± 0.19

APPENDIX H

RECOVERY OF HYDROCARBONS IN THE SCALLOP ADDUCTOR MUSCLE BY THE STEAM DISTILLATION PROCEDURE

The efficiency of the steam distillation technique for the recovery of hydrocarbons in the scallop adductor muscle was studied using a mixture of eight hydrocarbon standards. The eight hydrocarbons were representative of the major hydrocarbons that were present in the adductor muscle of scallops which had been exposed to condensate WSF.

About 50 g of adductor muscle from a control group of scallops was weighed into the distillation flask. The hydrocarbons were added to the flask as dilute solutions in ethanol of known concentration. The total volume of ethanol added was 100 µL. The amounts of hydrocarbons added are given in Table H.1. The spiked muscle was subjected to the steam distillation procedure with subsequent GLC analysis as described previously. The whole procedure (except the preparation of the ethanol solutions of hydrocarbons) was triplicated. The recovery results are given in Table H.1. The final correction factors, which incorporate the GLC system response correction factor, are given in Table H.2.

As can be seen in Table H.1, the percentage recoveries of the hydrocarbons were low. Methylnaphthalene showed the highest recovery of the eight hydrocarbons. The low recovery of hydrocarbons was apparently due to their high volatility. During the distillation, a major portion of the hydrocarbons must have escaped to the atmosphere through the water condenser of the distillation flask. Initially, another possibility was that the hydrocarbons remained in the distillation mixed with the tissue-water mixture. However, this was experimentally found (see below) not to be the reason for the low recovery of hydrocarbons.

Table H.1. Recovery of hydrocarbons from spiked scallop adductor muscle.

Hydrocarbon	Actual Amount Spiked (mg)	Experimental Recovery (mg)			% Recovery			Recovery Correction Factor[1]		
		Analysis 1	Analysis 2	Analysis 3	Analysis 1	Analysis 2	Analysis 3	Analysis 1	Analysis 2	Analysis 3
Benzene	.08786	.0098	.0083	.0078	11.15	9.49	8.89	8.97	10.54	11.25
Toluene	.08667	.0094	.0088	.0086	10.80	10.15	9.89	9.26	9.85	10.11
p-Xylene	.08607	.0164	.0173	.0155	19.05	20.08	18.01	5.25	4.98	5.55
o-Xylene	.08786	.0130	.0150	.0113	14.84	17.13	12.89	6.74	5.84	7.76
Propylbenzene	.10430	.0052	.0051	.0043	5.00	4.84	4.15	20.00	20.66	24.10
1,3,5-Trimethylbenzene	.08600	.0093	.0094	.0087	10.76	10.92	10.07	9.29	9.16	9.93
1,2,4-Trimethylbenzene	.08800	.0107	.0105	.0098	12.20	11.88	11.13	8.20	8.42	8.98
1-Methylnaphthalene	.10167	.0226	.0214	.0206	22.25	21.05	20.26	4.49	4.75	4.94

[1]Recovery Correction Factor = $\frac{100}{\% \text{ Recovery}}$

Table H.2. Recovery of hydrocarbons from spiked scallop adductor muscle - final correction factors.

Hydrocarbon	GLC System Response Correction Factor[1]	Final Correction Factor[2]			Mean ± Std. Deviation
		Analysis #1	Analysis #2	Analysis #3	
Benzene	0.91	8.16	9.59	10.24	9.30 ± 1.06
Toluene	0.96	8.89	9.46	9.71	9.37 ± 0.44
p-Xylene	0.91	4.78	4.53	5.05	4.80 ± 0.26
o-Xylene	0.91	6.13	5.31	7.06	6.17 ± 0.88
Propylbenzene	0.95	19.00	19.63	22.89	20.51 ± 2.09
1,3,5-Trimethylbenzene	0.93	8.64	8.52	9.24	8.80 ± 0.38
1,2,4-Trimethylbenzene	0.91	7.46	7.66	8.18	7.77 ± 0.37
1-Methylnaphthalene	---	4.49	4.75	4.94	4.73 ± 0.22

[1]See Appendix G for experimental determination of GLC system response correction factor.

[2]Final Correction Factor = $\frac{\text{Recovery Correction Factor (from Table H.1)}}{\text{GLC System Response Correction Factor}}$

The tissue sample and the water left in the distillation flask after the steam distillation was over were examined for any residual hydrocarbons. The tissue sample was saponified with KOH/EtOH and the non-saponifiable matter, which includes hydrocarbons, was extracted with diethyl ether. The diethyl ether extract was concentrated, subjected to thin-layer chromatography (TLC; developed in hexane, diethyl ether, acetic acid 85:15:1) and the hydrocarbon area was extracted. The recovered hydrocarbon TLC band was analyzed by GC. The chromatogram showed none of the above eight hydrocarbons.

The water sample was extracted with methylene chloride in a separatory funnel. The extract was concentrated and then examined by GLC for hydrocarbons. These results showed that the low recoveries of hydrocarbons from the steam distillation was due to the high volatility of the hydrocarbons, not due to solubility in the tissue-water mixture.

Although the recoveries were low, the percentage recoveries were reproducible, showing low variability (see Table H.1).

APPENDIX I

STATISTICAL CHART TRIANGLE TEST

Number of Taste Panelists	Number of Correct Answers Necessary to Establish Level of Significance		
	5%	1%	0.1%
7	5	6	7
8	6	7	8
9	6	7	8
10	7	8	9
11	7	8	9
12	8	9	10
13	8	9	10
14	9	10	11
15	9	10	12
16	10	11	12
17	10	11	13
18	10	12	13
19	11	12	14
20	11	13	14

Source: Larmond (1977)